GENETIC, PHYSIOLOGICAL AND MORPHOLOGICAL ASPECTS OF DWARF WHEAT

By

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Serenity Prayer:

"God grant me the serenity to accept the things I cannot change, change the things I can and the wisdom to know the difference".

Dedicated to the two most important people of my life, my loving mother, Hortense Madruga Pereira, and my mentor, Paul Leighton Pfahler, without whom the accomplishment and realization of this endeavor would not have been possible.

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Abstract of Dissertation Presented to the Graduate School
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GENETIC, PHYSIOLOGICAL AND MORPHOLOGICAL ASPECTS OF DWARF WHEAT

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The success of the "Green Revolution" which doubled wheat (*Triticum aestivum* L.) yields per unit area since 1960, can be partially attributed to the development and widespread use of semidwarf cultivars. Compared to normal or tall genotypes, the advantages of semidwarf genotypes include higher harvest index, yield plant^{-1} and yield ha^{-1} while the disadvantages are associated with reduced test weight and protein content but, more importantly, shorter coleoptiles which can result in unacceptable stands in the field. The present study examined the effect of a number of factors on germination and seedling characteristics of four homogenous and homozygous dwarf isolines [normal or tall height ($rht_1rht_1rht_2rht_2$) = T-0, semidwarf-1 ($Rht_1Rht_1rht_2rht_2$)= SD-1, semidwarf-2 ($rht_1rht_1Rht_2Rht_2$) =

SD-2, dwarf ($Rht_1Rht_1Rht_2Rht_2$) = D-12] in two genetic backgrounds ('Marfed' - a spring cultivar, 'Burt' - a winter cultivar). The reported gibberellic acid (GA) insensitivity caused by the presence of either the dominant Rht_1 or Rht_2 alleles was found to be partially overcome by lower germination temperatures and high GA concentrations (500 mg L^{-1} GA) in the germination medium. The addition of at least two other compounds [100 mg L^{-1} $Ca(NO_3)_2 \bullet 4H_2O$, 96.3 g L^{-1} polyethylene glycol 8000 (-0.156 Mpa osmotic potential)] to 500 mg L^{-1} GA in the germination medium resulted in a significant increase in coleoptile and first leaf length in T-0, SD-1 and SD-2 compared to 500 mg L^{-1} GA alone. Heritability studies indicated that environments in the form of seed production sites and germination temperatures were major factors influencing the expression of seedling characters. The response of the four isolines in each genetic background to light treatments differed. A highly significant negative relationship between coleoptile length and diameter was found. The results suggested that the problem of shorter coleoptile length in dwarfs can be at least partially overcome by genetic selection, preplant seed treatment with various compounds or a combination of both.

CHAPTER 1
INTRODUCTION

Wheat has been a very important crop since humankind's first successful attempts to initiate agriculture in southwestern Asia some 10,000 years ago (Zohary and Hoppf, 1993) and is still of prime significance presently (Evans et al., 1975). Wheat is used for food, feed, and industrial purposes, being second only to rice as a food crop (Briggle and Curtis, 1987). It is the most important staple food for more than one billion people in the world (Johansson, 1995) providing more than 20% of the calories for the world population of 5.3 billion people (Oleson, 1994).

Most of the wheat cultivars produced today are grouped together under the common name bread wheat which accounts for 95% of the world's production. The remaining 5% of cultivated varieties are allotetraploid durum wheats used for pasta, cookies, etc. (Oleson, 1994). Wheat is grown in six of the seven continents and in more than 100 countries. It can be grown in a wide array of environments ranging from Finland in the north to Argentina in the South. Wheat is the leading cereal grain produced in the world averaging 533 MMT annually which represents almost one-third of all cereal production (Oleson, 1994).

Since the 1960s, wheat production has more than doubled. This success of the 'Green Revolution' (Easterbrook, 1997) can be partially attributed to the development and use of semidwarf wheat cultivars. The vast majority of semidwarf wheat varieties covering more than half the world's wheat acreage now carry either of the dominant dwarf alleles at the dwarfing loci (Worland and Petrovic, 1988; Allan, 1980b, 1989) from the Japanese cultivar 'Norin 10' (Allan, 1970). Alleles for dwarfing in wheat are identified by the symbol *Rht* (reduced height). Rht_1, Rht_2 and Rht_3 are the three dominant alleles responsible for the dwarf phenotype in wheat. The first two, are the most commonly used in wheat breeding programs. The Rht_3 allele, has an extremely intense dwarfing effect that is a disadvantage agronomically. The genetic basis of Norin 10 has been clarified (Gale and Marshall, 1976; Gale et al., 1981). Rht_1 and Rht_3 are alleles at a locus on chromosome 4BS (nomenclature post 7th International Wheat Genetics Symposium, 1988) and Rht_2 is at a homeoallelic locus on chromosome 4DS (Pinthus et al., 1989; Youssefian et al., 1992a and literature cited therein). Tall or standard height wheat cultivars, are homozygous recessive for dwarfing at the two dwarf loci ($rht_1rht_1rht_2rht_2$), while semidwarf lines can have the dominant dwarf allele either at genome B or D ($Rht_1Rht_1rht_2rht_2$ or $rht_1rht_1Rht_2Rht_2$, respectively); the dwarf lines (very short) have dominant alleles ($Rht_1Rht_1Rht_2Rht_2$)in both genome sources, B and D.

The use of dwarfing in wheat has advantages and disadvantages. On the positive side, dwarf genotypes carrying the alleles Rht_1 and/or Rht_2 have significantly higher harvest index, yield plant^{-1}, tiller number meter^{-2}, grain number ear^{-1} and longer spikes than tall types in hexaploid wheat (McClung et al., 1986), with the higher yielding potential attributed to a greater leaf conductance to CO_2 (Fischer et al., 1981). Despite the widespread acceptance of Rht_1 and Rht_2, they have been associated with a number of undesirable pleiotropic effects. These include gibberellic acid (GA) insensitivity, reduced test and seed weight, reduction in protein content and reduced coleoptile length relative to the tall lines (Allan, 1989), which, in some genetic backgrounds, causes establishment problems in dry seed beds (Allan, 1980a). Other pleiotropic effects are: cell size and number, root weight, leaf size, seed yield and yield components, biomass, harvest index (Allan, 1989) and disease reaction (Gale and Youssefian, 1985). The reduced test and seed weight of the semidwarf lines can be an important quality factor because it may result in lower milling extraction percentages and semolina yields relative to tall lines; the low grain protein concentration is only observable on an individual grain basis, but it is comparable with talls in a yearly and area basis (Gale, 1979).

A striking property of gibberellic acid (GA) is its capacity to greatly stimulate the growth of dwarf plants in certain species which grow at rates similar to normal plants following GA treatment. Wheat seedlings (coleoptiles and first leaves) completely homozygous and recessive for the dwarf alleles elongate extensively in the presence of GA, while those that have at least one dominant dwarf allele present, are insensitive to GA application (McClung et al., 1986). Due to that insensitivity, GA is widely used in early generations of breeding programs when semidwarf alleles are incorporated into adapted tall or standard lines. Because reduction in stands is a serious limitation to the use of dwarf wheat, further studies are needed to evaluate the use of GA as a seed treatment to overcome the short coleoptile length (Allan et al., 1961).

The objectives of this study were to examine various physiological and morphological aspects of dwarfing on the coleoptile, first leaf and root length of wheat seedlings grown under laboratory conditions. The effects of exogenous application of GA, other growth regulators and various organic and inorganic compounds in the germination medium, were examined under various temperature and light regimes. The effect of various seed production environments on the expression of dwarfing was determined using heritability procedures. In addition, the effect of dwarfing on coleoptile dimensions was studied.

CHAPTER 2
LITERATURE REVIEW

Seedling Characteristics

Studies have shown that the germination percentage in dwarf wheat initially increased with an increase in seeding depth from 25 to 55 mm and decreased at seed depths greater than 55 mm (Singh et al., 1991). At equivalent depths of planting which exceeded the maximum length of coleoptiles and under moderate soil compaction, cultivars carrying the dwarf *Rht* allele were at a biomechanical disadvantage as far as seedling establishment was concerned (Niclas and Paolillo, 1990). Final coleoptile length from seeds of different genotypes germinated in the dark provided an indication of the planting depth limits, which would produce an acceptable final stand in the field (Wiedenroth et al., 1990).

Observations that poor seedling emergence was a major shortcoming of semidwarf winter wheat selections in the Pacific northwest led to research concerning emergence rate and coleoptile length. The difficulty in getting proper stands when dwarf seeds were planted deeply, was due to the reduced coleoptile length and slow seedling growth rate, all contributing to the poor stand establishment (Allan, 1980a;

Allan et al., 1961; Niclas and Paolillo, 1990; Parodi et al., 1970). Thousands of Daruma-type semidwarf lines were analyzed and all had short coleoptiles and slow seedling growth rates (Allan, 1980a). Since then, attention has been focused on the relationship between coleoptile length and seedling emergence and establishment in wheat. The effects of GA on seedling emergence and coleoptile length of slow and fast-emerging wheat varieties of dwarfs and talls, respectively, have been also focus of attention (Allan et al., 1961). Coleoptile length is associated with rate of seedling emergence, which gives talls some advantages over their dwarf counterparts, by germinating faster. The normal height or tall seedlings produce longer coleoptiles and longer subcrown internodes than *Rht* seedlings (Niclas and Paolillo, 1990). Studies on the analysis of coleoptile length in wheat reported high narrow sense heritability values, suggesting a predominance of additive gene action and low environmental influence (Parodi et al., 1970). Coleoptile measurements are also very useful in predicting final plant height in semidwarf breeding programs, because extremely tall or short lines are accurately identified by the length of their coleoptiles (Allan et al., 1962). The genetic mechanisms that control coleoptile length and plant height appeared pleiotropic in most varieties (Gale and Youssefian, 1983).

The "GA-Insensitive" Term

Some 50 years of research have established that development in plants is associated with plant growth substances (Trewavas, 1982). Exogenous application of hormones is one of the most common methods based on the concept of replacement of the endogenous, naturally-occurring hormone by an exogenous hormone, the level of which may be controlled and its effect monitored (Zeroni and Hall, 1980). The ability of a tissue to respond to a hormone is conditioned by its intrinsic capacity for response (or secretion) and the rapidity with which maximum elongation rate is achieved (Stoddart, 1986). The simple interpretation of sensitivity is that it represents the presence or absence of specific receptor proteins. Since these receptors are membrane-located, alterations in factors which change or alter membrane structures, such as ion levels and fluxes, electrical potentials, pressure, lipids, membrane proteins and hydrophilic molecules, will likewise alter the interaction of the growth substance with its receptor; thus changing sensitivity (Trewavas, 1982).

Rht$_1$ and *Rht*$_2$ are unusual among the twenty or so minor alleles affecting plant height in wheat in that they are associated with an insensitive reaction to applied GA (Youssefian et al., 1992a). Similar results were observed in the 3 diploid rye genotypes that Borner (1991) examined in which the recessive dwarf alleles were GA-insensitive. On the other hand, Borner et al. (1993) reported two different recessive alleles in diploid rye genotypes for dwarfism which are sensitive to GA. Usually, in species where dwarfs respond to GA application, dwarfing is recessive. Genetic and physiological evidence in rye indicated that the two recessive alleles for dwarfism are at different loci and are unrelated to the GA-insensitive *Rht* alleles in hexaploid bread wheat. Those dwarf plants that elongate normally in response to exogenously-supplied GA are likely to have a lesion in a GA biosynthetic gene, or in the regulation of such a gene (Chasan, 1995). Other compounds, such as the substituted phthalimide AC 94,377 ([1-(3-chlorophthalimido) cyclohexanecarboxamide]) behave similarly to the gibberellins in a number of plant systems. The AC 94,377 compound can induce elongation in normal (tall) height wheat and barley (*Hordeum vulgare*) seedlings, but wheat lines containing *Rht* alleles are insensitive (Rodaway et al., 1991).

Temperature effects

In general, the coleoptile length is highly affected by temperature. Kaufman et al. (1968) reported that, in *Avena sativa* L., high temperatures greatly reduced coleoptile length which, in turn, was also affected by depth of planting and soil compaction. Allan et al. (1962) found coleoptile length, emergence-rate index and plant height to be positively correlated at 10 and 32 C. In essence, the relation between coleoptile length and emergence-rate index paralleled the relation between plant height and emergence rate index (Allan et al., 1962; Parodi et al., 1970). In addition, at 32 C, coleoptile length and first-leaf growth measurements were found to produce the highest correlations with plant height (Allan et al., 1965). Allan et al. (1962) also related poor seedling emergence of some wheat selections to high soil temperatures. His results showed that maximum coleoptile elongation was obtained at 16 C, whereas considerable reduction in coleoptile length was observed with increases of temperatures to 27 C. The mean field emergence-rate indexes of dwarf selections grown at 10 and 32 C were very low. The lower temperature was most suitable for emergence of the semidwarfs, and the high temperature slightly better for the normal height groups (Allan et al., 1962). The authors concluded that, because of these interactions, the optimum temperature at which to grow seedlings was impossible to determine.

Gibberellic acid (GA) effects

GA application to intact plants or excised sections of plants generally resulted in substantial promotion of growth by extension (Zeroni and Hall, 1980). GA modifies the climatic control of plant growth; seed treated with GA gives earlier and more uniform emergence which results in better stands in such crops as beans, peas, cotton, and certain small grains (Radley, 1970; Wittwer and Bukovac, 1958). However, GA treatment did not increase dwarf wheat coleoptile length but promoted uniform emergence under greenhouse conditions; therefore, the increased rate of emergence due to GA treatment appeared to be independent of the coleoptile length (Allan et al., 1961).

Under deep seeding conditions (greenhouse test), GA treatment had little or no effect on dwarf wheat coleoptile length (Allan et al., 1961). It seemed that the two characters, GA-insensitivity and coleoptile length, remained completely associated even in segregating families (Gale and Marshall, 1975). These results also showed complete association between endosperm GA-insensitivity and coleoptile length. This could mean that physiological studies with the aleurone layers of dwarf seeds might shed some light in future studies involving the use of hormones, such as GA, and coleoptile length of dwarfs and semidwarf wheat selections. However, Stoddart and Lloyd (1986) disagreed with this point of view, and their results showed that after pre-treating the seed with GA at 5 C up to 10 days, no differences in the GA-response of first, second and third leaf were obtained at 20-25 C growing temperature. Their conclusion was that the GA-response mechanism between aleurones and leaf meristems are different.

Temperature and GA effects

A positive correlation between temperature and first leaf elongation was observed in tall (*$rht_1rht_1rht_2rht_2$*) seedlings without GA application. When *Rht_1* and *Rht_2* were present, the effect of increasing the temperature on first leaf elongation was in most cases less than half the effect observed in tall (*rht*) genotypes (Pinthus et al., 1989). At 11 C, *$rht_1rht_1rht_2rht_2$*, *$Rht_1Rht_1rht_2rht_2$*, *$rht_1rht_1Rht_2Rht_2$* and, *$Rht_1Rht_1Rht_2Rht_2$* seedlings had the same first leaf length. When GA_3 was applied to the seedlings, the length of the first leaf of normal or tall increased 110 to 170% but the GA sensitivity was not affected by temperature in this genotype. A different scenario was observed with *$Rht_1Rht_1rht_2rht_2$*, *$rht_1rht_1Rht_2Rht_2$* and *$Rht_1Rht_1Rht_2Rht_2$*. The response to applied GA in these genotypes was very temperature dependent and was most responsive at low rather than at high temperatures. In rye, the stems of recessive dwarf lines were 45-50% as responsive to applied GA as the tall lines at 20 C but the absolute GA-responsiveness of the dwarf rye was greater at 10 than at 20 C (Borner et al., 1993).

Physiology of Growth and the Role of GA

A number of reports indicated that the GA-mediated elongation of shoots of various species occurs as a result of an increase in mitotic frequency (cell number), cell enlargement, or both (Wu et al., 1993). In *Avena*, cell elongation started extremely early in internode development when the internode was just 2-3 mm long. Cell division and cell elongation are processes that clearly overlap during early (30-40 mm) stages of internodal development (Kaufman et al., 1965). Therefore, most of the linear growth observed was the result of cell elongation.

In normal height *Avena*, growth promoted by GA in excised stem segments is by far the greatest of any excised tissue. It has been shown in semidwarf and dwarf wheat that GA affects growth by enhancing both cell division and cell elongation (Hoogendoorn et al., 1990; Keyes et al., 1989). Stuart and Jones (1977) reported that an increase in cell turgor was not the principal driving force for growth in hypocotyl sections of *Lactuca sativa*. Their conclusion was that whether GA stimulates cell elongation by increasing turgor pressure or changing cell wall extensibility was not known. Cleland et al.(1968) did not measure cell wall plasticity, but the differences in osmotic potential between hypocotyls of tall and dwarf cucumber suggest that the GA_1 turnover may also influence cell extension by altering turgor pressure. GA_1 is the only GA causing internode elongation *per se*. Other GAs, like exogenously-applied GA_3, appear active because they are converted to GA_1 in the assay plants of pea, maize and rice (Graebe, 1987) and probably wheat too. In the young culm internode of *Avena*, the intercalary meristem is actively dividing during a third of the total growing period, after which cell division stops and cell elongation is the sole cause of elongation (Kaufman et al., 1965). GA added at the time of maximum cell division shortened the period of active division and increased the mean cell length of cells, but had no distinct effect on the number of cells. GA-stimulated growth of *Avena* stem segments was also shown to be accompanied by large changes in cell wall plasticity (Adams et al., 1975). Most studies examined epidermal cells of culms, but similar trends were apparent for pith cells. Cell extension in the GA-treated *Avena* internodes occurred simultaneous to an increase in cell wall plasticity. This response in addition to changes in turgor pressure and concurrent cytoplasmic protein synthesis were considered to be the driving force behind cell elongation (Cleland, 1971; Stoddart, 1986). GAs may act by virtue of an association with cellular membrane. The most pronounced effect of GA in stimulating cell elongation is an increase in cell wall plasticity, which occurs at the initiation of extension growth (Adams et al., 1975).

In cotyledonary tissue, cytokinins may induce an increase in cell size. Zeroni and Hall (1980) reported that, in most cases, cytokinins inhibit endogenous or auxin-induced growth in stem tissue. It has been frequently observed in soybean hypocotyls that simultaneous addition of auxin and cytokinins inhibit extension, but not an increase in fresh weight since isodiametric extension is promoted (Zeroni and Hall, 1980). Kaufman (1965) working with *Avena* coleoptiles showed that indole acetic acid (IAA) and GA have remarkably different effects on intercalary growth in *Avena* internodes. IAA strongly promoted longitudinal growth in light, while suppressing growth in dark. However, GA at comparable concentrations accelerated longitudinal growth of *Avena* internodes in both light and dark but did not increase internode dry weight at several concentrations (Kaufman, 1965).

Abcissic acid (ABA) will normally inhibit overall extension growth, although at high concentrations, extension may be transitorily promoted as a consequence of low pH ("acid growth theory") (Zeroni and Hall, 1980; Varty et al., 1982).

Physiology of Dwarfing

Limited knowledge of the physiology of dwarfism in wheat is available. The mechanism by which Rht_1 and Rht_2 alleles are involved in the cell extension, is apparently in decreasing cell wall extensibility or by the withholding of applied GA (Keyes et al., 1989; 1990). According to Gale and Marshall (1973), gibberellin metabolism is almost certainly involved. On the other hand, Stoddart (1984) reported that the insensitivity trait must be related to the ability of the dwarfs to interact with GA, thus suggesting that GA metabolism was not an essential feature of the biological action of GA in wheat. The lack of GA response in a tissue can be attributed to many causes, such as the presence of inhibitors and a general slowdown in cellular metabolism (Borner, 1991).

Studies on *Rht*$_3$ dwarf wheat showed no reduction in cellular metabolism such as respiration rate, protein content, rate of protein synthesis, and uptake of amino acids. The content of ATP was even higher in *Rht*$_3$ dwarfs than in talls although all of the gibberellin-mediated physiological processes that were studied, including leaf elongation, synthesis and release of hydrolytic enzymes and secretion of phosphate ions and reducing sugars in aleurone layers, were retarded in the same dwarfs (Ho et al., 1981). Unlike the seedlings, the flag leaf of mature dwarf wheat plants appeared to contain higher sugar level than those of talls (Radley, 1970). When cotyledons of germinating normal-height *Vigna mungo* were examined after exogenous application of plant hormone and growth regulators, levels of α-amylase activity and content were reduced by high concentrations of glucose and sucrose. It was suggested that their effect was caused mostly by osmotic stress and partly by end-product repression (Taneyama et al., 1995), however, the level of α-amylase was nearly twice that in controls after 1-10 μM GA_3 had been applied to the cotyledons. In addition, 0.1 mM kinetin, 0.1 mM dimethylamine salt of 2,4-

dichlorophenoxyacetic acid (2,4-D) and 0.1-0.5 mM naphthalene acetic acid (NAA) also increased the α-amylase level by 34 to 66% compared to the control (Taneyama et al., 1995).

The invertase enzyme, which hydrolyses sucrose into glucose and fructose, has been implicated in starch, cellulose and ATP biosynthesis, and in maintenance of turgor pressure. Invertase was higher in GA_3-treated dwarf pea stems than in untreated plants (Wu et al., 1993). In several species, a growth response to GA_3 was shown to be accompanied by an increase in invertase, for example in *Avena* internodes (Kaufman et al., 1968).

According to Ho et al. (1981), talls and dwarfs have the same endogenous uptake and metabolism of gibberellins as inhibitors such as ABA. In tall wheat, the short-term reduction in cell-wall extensibility induced by low temperature was reversed by applied GA_3 and the relative responsiveness was greater at lower than at higher temperatures (Stoddart, 1986). He found part of the active GA in the pectin fraction of the cell wall which is considered to be related to the stiffness of the wall.

Differences in Anatomy Related to Plant Height of Tall and Dwarf Wheat

From a detailed physiological and biochemical analysis of a single-gene recessive dwarf mutant, it was established that GA_1 is the active compound responsible for increased leaf sheath and stem internode elongation (Phinney, 1984; Chasan, 1995). GA insensitivity conferred by *Rht* (the dominant dwarf allele in wheat) limits the rate of growth. Shorter cells were present because there is no effect of *Rht* on cell division in leaves that compensated for reduced wall extension at the cellular level (Keyes et al., 1989). In dwarfs, reduced cell size was associated with reduced (more negative) osmotic potential of the cell solutes (Hoogendoorn et al., 1990). The number of *Rht* alleles (only one or two) present, increased cell length and reduced cell width (Keyes et al., 1989). Most of the variability (r^2=0.80) for cell length was the result of a linear response to the number of *Rht* alleles regardless of genetic background. Cell number and duration of elongation were independent of *Rht* number. It is believed that the *Rht* alleles' primary action involves modification of the plant's GA response system (Lenton et al., 1987) which reduced the cell size of vegetative tissues. It was showed that peduncles and first leaves of Rht_1 and Rht_2 genotypes had significantly reduced cell lengths, while the number of cells were only slightly reduced (Hoogendoorn et al., 1990; Keyes et al., 1989).

The length of an internode is determined by the number and length of its cells (Nilson et al., 1957). In wheat containing the *Rht* alleles, the parenchyma cells of each internode were shorter than those of corresponding tall lines. Parenchyma and epidermal cells were of the same length in dwarfs while, in talls, epidermal cells were somewhat longer than parenchyma cells (Nilson et al., 1957; Keyes et al., 1989). When exogenous GA was applied to the tall sensitive lines, increases in leaf length were associated with increases in cell length (Hoogendoorn et al., 1990). The similarity between talls (rht_3) and dwarfs (Rht_3) on a dry-weight basis indicated that the reduced size was caused by smaller, rather than fewer cells. This was confirmed by measurements of epidermal cell length of the first leaf (Stoddart, 1984; Tonkinson et al., 1995). Leaves of dwarf seedlings had shorter epidermal cells; number of cells per unit length in talls and dwarfs matched the observed relationship in overall length. Thus, the genetic differences appeared to be principally expressed in terms of cell enlargement rather than cell division (Stoddart, 1984).

Endogenous GA Analysis

It has long been known that endogenous GA often replaced the need for environmental stimuli such as low temperatures (Karssen et al., 1989). Therefore, it has been hypothesized that endogenous GA accumulates in seeds in response to such stimuli. Maximum gibberellin levels were found in young leaves under high levels of nutrition. Germinating seeds, green seedlings and stems of dwarf wheat cultivars contain more, not less, endogenous GA than tall forms (Radley, 1970). High levels of endogenous gibberellin in a particular wheat variety do not necessarily indicate that the plant will be tall (Lenton et al., 1987; Rodaway et al., 1991). Actually, tall varieties are more likely to respond to exogenously-applied gibberellin compared to dwarfs.

When only the tall genotypes were considered, the GA levels were highest in seedlings germinated at 20 C, lowest when seedlings were germinated at 2 C, and intermediate when seedlings were germinated at 2 C and then transferred to 20 C (Reid et al., 1974). Similarly, Pinthus et al. (1989) compared *rht* with *Rht* genotypes and reported on the endogenous GA levels. At 25 C, the GA concentration was the lowest in the *rht* genotype, was high in $Rht_1Rht_1rht_2rht_2$ and $rht_1rht_1Rht_2Rht_2$, and even higher in $Rht_1Rht_1Rht_2Rht_2$. At temperatures lower than 10 C, the same endogenous GA concentration was found in all the genotypes referred above (Appleford and Lenton, 1991; Pinthus et al., 1989).

Any GA species will be converted to GA_1 in the plant, because the latter is essential for shoot elongation. GA_1 in dwarf wheat accumulates in proportion to the potency of the dwarfing allele (Appleford and Lenton, 1991; Pinthus et al., 1989). The concentration of biologically-active GA in cultivars containing Rht_1, Rht_2, Rht_1Rht_2 and Rht_3 alleles, was much greater than that of tall cultivars (Radley, 1970; Stoddart, 1984). The increase in GA_1 in the dwarf lines has been explained in terms of reduced metabolism in nonexpanding tissue (Graebe, 1987). In a similar fashion, genotypes that are responsive to an increase in temperature by producing more GA_1, were also responsive to applied GA_3 (Pinthus et al., 1989). The responses are the greatest in $rht_1rht_1rht_2rht_2$, moderate in $Rht_1Rht_1rht_2rht_2$ and $rht_1rht_1Rht_2Rht_2$, and only slight in $Rht_1Rht_1Rht_2Rht_2$. Radley (1970) demonstrated that the semidwarfs had comparatively high levels of free endogenous GA_1 and/or GA_3 and suggested that this may be due to the inability of these genotypes to metabolize the hormone. It is therefore likely that the high levels of endogenous GA, insensitivity to applied GA and dwarfism are related characteristics in wheat (Gale and Marshall, 1973).

Mature Dwarf Tissue Physiology

Plant stature and leaf length are reduced by 50% in the presence of the Rht_1 and/or Rht_2 allele(s), but root and culm dry weights are much less affected (Stoddart, 1984).

GA application

Gale and Gregory (1977) found that, in durum wheat, the Rht_1 allele is completely linked to the GA insensitivity allele (Gai_1). The first leaf of seedlings homozygous for Rht_1 were insensitive to exogenously-applied GA, while homozygous rht_1 seedlings elongated extensively in the presence of the hormone. The same phenomena was observed by

Allan et al. (1959) in three semidwarf bread wheat cultivars which showed no significant increase in height when different GA concentrations and several consecutive injections were applied. Their results also showed that the tall cultivars (standard approximately 120 cm) had significant increases in height compared to controls. This method can be used by breeders to effectively screen segregating progeny for presence or absence of the allele during the first leaf seedling stage (Gale and Gregory, 1977; McClung et al., 1986).

Semidwarfs treated with GA_3 were 38% taller than their respective controls in the Gale and Marshall (1973) studies, but those results were not significant. Also, studies conducted by Allan et al. (1959) showed that wheat semidwarf genotypes had no significant reaction with exogenously-applied GA at 5 ppm. Gale and Marshall (1973) however, reported dwarfs producing more tillers in response to GA treatment and tall producing fewer tillers.

Besides culm and leaf elongation, GA also affected dry weight and flowering time of tall cultivars. Genotypes carrying *Rht* alleles were relatively insensitive to applied GA_3, however (Gale and Marshall, 1973; Radley, 1970). GA treatment lowered the dry weights of both roots and shoots in tall cultivars and was associated with no significant reduction in the dry weight of dwarfs (Gale and Marshall, 1973). The percentage reductions in dry-matter correlated closely with the percentage increase in total stem lengths (Youssefian et al., 1992b).

Temperature effect. In near-isogenic dwarf wheat lines carrying all possible homozygous combinations of the *Rht*$_1$, *Rht*$_2$ and *Rht*$_3$ alleles, it was shown that temperature affected the phenotypic expression of the dwarfing alleles and the degree of responsiveness of the culm tissues to applied GA_3 (Appleford and Lenton, 1991). In a study with *Rht* wheat and *Arabidopsis thaliana,* faster germination was obtained as a response when GA_3 was applied at low temperatures (Karssen et al., 1989). The absolute responsiveness was greater at 10 C than at 20 C and, since exogenous GA_1 (plus GA_3) content was similar, it seems reasonable to conclude that endogenous GA may be more sensitive regulators of leaf expansion at lower than at higher temperatures (Appleford and Lenton, 1991).

The term 'GA-insensitivity', which is usually associated with these *Rht* alleles, should not be considered as being absolute but dependent on the growing temperature (Pinthus et al., 1989). Culm and leaf elongation of *Rht* genotypes are insensitive to applied GA_3 (Gale and Marshall, 1973; Pinthus et al., 1989; Radley, 1970) at temperatures of about 20-25 C (Pinthus et al., 1989). At lower temperatures (11 C), leaf growth of *Rht*$_1$ and *Rht*$_2$ were much more responsive to applied GA_3 than tall genotypes. It is reasonable to assume that in the *Rht* genotypes grown at

10 C, the GA_1 content was suboptimal for maximum growth, since it was similar to that of *rht*. Thus, the potential to respond to applied GA_3 was greater at lower temperatures although the magnitude of the response was determined by the doses of the *Rht* allele (Pinthus et al., 1989).

Explanations for GA insensitivity in mature tissue

The scheme proposed by Youssefian et al. (1992b) indicated that the mode of action of the *Rht* alleles is associated with a block in GA_1 and/or GA_3 utilization in the vegetative tissue, which reduces cell elongation (Hoogendoorn et al., 1990; Nilson et al., 1957; Keyes et al., 1989) and which, in turn, reduces stem elongation only in vegetative tissues. Allan et al. (1959) concluded that semidwarf and dwarf characteristics of wheat were not due to a specific lack of GA nor to a substance replaceable by GA, but instead the dwarfism could result from the inability of wheat to use GA or related growth substances to an appreciable extent. Baroncelli et al. (1988) attributed the different responses of talls and dwarfs to GA application, as being probably the result of different endogenous GA levels. However, earlier research reported that there were no differences in endogenous GA levels between talls and dwarfs (Gale and Marshall, 1976). Hoogendoorn et al. (1990) suggested that the physiological availability of GA limits cell extension in dwarfs. This means that the primary effects of the GA-insensitive *Rht* genes is to reduce the efficiency of the mechanism by which GA_1 is actively metabolized in elongating tissues. That results in an accumulation of unused GA_1 early in the GA pathway in the *Rht* genotypes.

Singh and Paleg (1984b) reported no effect of low temperature treatment on GA_3 sensitivity in wheat in the absence of exogenously-applied $GA_{3.}$ The increase in GA_3 sensitivity in semidwarf tissues due to the low temperature treatment was assumed to be due to via an increase in the levels of endogenous gibberellins. Gale and Marshall (1975) reported the insensitivity response was associated with high levels of GA_1 and/or GA_3 in seedlings, mature stems, and leaf material. Conversely, in the germinating seed, the lack of response was associated with normal GA production. The accumulation of hormone seems to be a consequence of non-utilization.

According to Stoddart (1984), leaves of dwarfs metabolize exogenously applied GA_1 at a slower rate than semidwarfs, and semidwarfs metabolize at slower rates than talls. The effectiveness of GA_1 in promoting growth of tall seedlings indicates that the insensitivity trait must be related to the ability of the dwarfs to interact with this GA and is not related to metabolism. The authors suggested that consideration of insensitive dwarfism could be more profitably concentrated on studies of the subcellular associations of the biologically-active endogenous GA structures. Another explanation given by Gale and Gregory (1977) is that dwarf alleles probably operate by restricting the turnover of the hormone in the plant, and one manifestation of this restriction is the GA insensitive reaction.

Explanation for the observed higher yields

The effects of the *Rht* alleles on cell length rather than on numbers of cells would explain why, under high yielding conditions, these alleles have been found to reduce plant height, but not biomass (Hoogendoorn et al., 1990). The two most convincing hypotheses put forward to explain the increased seed numbers are based on differences in either morphogenesis, or the proportion of total dry-matter partitioned to the apex (Youssefian et al., 1992a). From a physiological point of view, the effects of the GA-insensitive dwarfing alleles Rht_1 and Rht_2 on the growth and partitioning of dry-matter in wheat are to reduce the rate of stem elongation and vegetative dry-matter accumulation (Youssefian et al., 1992b). Consequently, from very early in development, there is reduced competition by the stems for assimilates leading to a greater proportion of dry-mass being partitioned to the *Rht* genotype spikes. Such partitioning results in a greater number of competent florets, which also have a greater mass per carpel at anthesis. Both these features are considered to favor increased seed set, which is characteristic of dwarfs (Youssefian et al., 1992b). The *rht* and *Rht* genotypes have different harvest indices. From the very early stages of growth, relatively greater amounts of total dry-matter were partitioned to spikes of genotypes carrying one of the dominant dwarf alleles than to spikes of the talls. So, the presence of the *Rht* alleles is associated with a greater proportion of dry-matter going to the spike (Youssefian et al., 1992b).

The GA-insensitive dwarfing alleles do not affect development events in either the growing apex or vegetative tissue (Youssefian et al., 1992a). The dwarfs do not elongate significantly after GA application, but produce more tillers as a consequence (Youssefian et al., 1992a). The usual reaction of intact plants to applied GA is to increase apical dominance or, as an indirect GA effect, via the induction of auxin synthesis or repression of auxin breakdown. They also found that lateral growth in decapitated plants was stimulated when GA was applied. The similarity between the action of GA on dwarf wheat and in decapitation experiments, led to the conclusion that the metabolism of GA is blocked before the GA-auxin link allowing the applied GA to stimulate lateral growth by a different and unaffected pathway. According to Gale and Marshall (1973) this explanation would account for the higher tiller numbers normally found in the dwarfs since they apparently utilize endogenous GA in this way.

Physiological Studies on Dwarf Aleurone Layer

The relationship between gibberellins and gibberellin-receptors in plant height expression in wheat and barley is not completely understood (Rodaway et al., 1991). GA-mediated elongation of first leaf still occurred in tall seedlings in which the endosperm had been removed (Ho et al., 1981). This finding indicates that the GA-mediated elongation in dwarf wheat is independent of the seeds' ability to mobilize endosperm.

GA Application

Dwarf wheats differ from tall in their lack of response to applied GA. In dwarf wheat, as in most cereals, the aleurone layer surrounding the endosperm in the seed responds either to exogenous GA or to GA supplied by the embryo during germination by producing the enzyme α-amylase (Gale and Marshall, 1975; Eastwood et al., 1969). The embryo is capable of producing those gibberellins (GA_1 and GA_3, the most active forms for the induction of growth in various species) that can induce α-amylase production in the aleurone layer (Großelindemann et al., 1992). Radley (1970) showed that a number of spring semidwarfs responded only slightly to GA in height, but responded normally to GA in the induction of α-amylase synthesis in the aleurone layer of seeds. Tall and dwarf isolated aleurone layers produce the same amount of α-amylase in the absence of GA_3 (Ho et al., 1981). With the exogenous application of 1 μM GA_3, dwarf aleurone layers increased their α-amylase production by 3-fold, whereas there was more than 100-fold enhancement in the aleurone layers of their tall counterparts. The release of α-amylase to the medium was also enhanced by GA in the talls but not in dwarf genotypes.

The role of calcium

The role of calcium (Ca^{2+}) in hormone-induced cell elongation is not clearly understood. The effect of GA_3 might be one of reducing the Ca^{2+} activity in the cell wall, since results indicated that other treatments which promote growth by removal of Ca^{2+}, are in fact removing Ca^{2+} from the wall (Moll and Jones, 1981). A study in barley aleurone tissue demonstrated that the synthesis and release of α-amylase requires both GA and Ca^{2+} (Carbonell and Jones, 1984). Ca^{2+} facilitates the passage of α-amylase through the cell wall via an interaction between the ion and an exchangeable site in the wall. In lettuce, gibberellin controls extension growth of the hypocotyls sections by regulating the uptake of Ca^{2+} in the hypocotyl cells (Moll and Jones, 1981).

GA and temperature effects

Low temperatures have been shown to alter numerous physiological responses of plant tissues. There are numerous examples of interactions between gibberellin responses and low temperature in dwarf wheat (Singh and Paleg, 1984 a, b, c, 1986c). In the absence of exogenous GA_3, preincubation time or temperature have no effect on the sensitivity of the isolated dwarf aleurone tissue to GA. When GA_3 is applied exogenously, low temperature (5-10 C) preincubation increases the GA_3 response of the aleurone tissue 5-12 fold. The optimum GA_3 concentration (0.1 µg ml^{-1}) for the α-amylase response remains unchanged, irrespective of preincubation temperature and time, although 24 h preincubation at 5 C seems to maximize GA_3 sensitivity (Singh and Paleg, 1984b).

Aleurone layers of mature seeds of wheat cultivars carrying, *Rht*$_1$, *Rht*$_2$, or *Rht*$_3$ alleles became responsive to applied GA_3 after preincubation at 5 C for 20 h (Singh and Paleg, 1984 a, b). Low temperature preincubation considerably increased the GA_3 sensitivity of seeds having one of the *Rht* alleles, but no increase in sensitivity was observed on the seeds with *rht* alleles (Singh and Paleg, 1984b). In other words, isolated dwarf wheat aleurones and mature seeds can be made responsive to GA at low preincubation temperature treatments (Singh and Paleg, 1984a, 1986a). The increase in sensitivity was monitored by determining the amounts of α-amylase produced by the aleurone tissue in response to exogenous GA_3 (Singh and Paleg, 1984b). The similarity of the effect of low temperature on the GA_3 sensitivity of both isolated aleurone and deembryonated dwarf seeds eliminated the involvement of the embryo and the endosperm in perceiving or responding to the low temperature-induced GA_3 sensitivity.

GA, temperature and IAA effects

Dwarf wheat aleurones can respond to GA_3 at low temperatures (Singh and Paleg, 1984a; 1985; 1986a) and can also be rendered GA_3-sensitive by preincubation with indoleacetic acid (IAA). It appears that the control of cell extension is based on IAA (Zeroni and Hall, 1980). The effects of low temperature preincubation and IAA pretreatment in enhancing the subsequent GA_3 response are not synergistic. Both can produce similarly increased GA_3 responsiveness of dwarf aleurone tissue when present alone or together during the preincubation period (Singh and Paleg, 1986a). IAA alone during preincubation can duplicate the low temperature-induced enhancement of the GA_3 responsiveness of the dwarf aleurone tissue (Singh and Paleg, 1985; 1986a). When incubation was conducted at 30 C without IAA, no GA sensitivity was detected, but with added IAA a dramatic increase in the sensitivity of the aleurone tissue was observed. On the other hand, incubation of deembryonated seeds exclusively with GA_3 at 30 C did not influence GA_3 response while, IAA had no effect on the GA_3 sensitivity of the *rht* aleurone tissue.

Explanations for GA Insensitivity at the Aleurone Level

Several theories/hypothesis are given to explain GA insensitivity in wheat. It has been suggested that the genetic dysfunction of a *Rht* dwarf wheat is located in the aleurone tissue, since GA_3 released from the embryos of insensitive dwarf varieties during germination, is similar to that of sensitive tall genotypes. Embryos of one genotype transplanted and grown on the endosperm of another showed that lack of response was a function only of the endosperm tissue (Gale and Marshall, 1975). They concluded that since GA-insensitive alleles are genetically dominant and, as a result, probably 'active,' they encode a product that reduced height by blocking GA response. Devi et al. (1994) believed that the action of the dwarf mutation may also be indirectly related to GA action and that GA receptors or steps in the signal transduction pathway may also be affected in dwarf wheat plants.

The low temperature treatment of deembryonated seeds "cures" or circumvents the genetic lesion present in the aleurone tissue of fully mature seeds containing at least one *Rht* allele. Low temperature is involved in an increase in GA_3-effective receptor sites (Gale and Marshall, 1975; Singh and Paleg, 1984a). This would explain why low temperature-induced GA_3 sensitivity is GA_3 concentration-dependent. Low GA_3 concentration (0.01 µg ml^{-1}) is not sufficient to saturate the increased number of receptor sites produced as a result of low temperature (Gale and Marshall, 1975). This is very similar to the results reported by Ho et al. (1981). They found that a low GA_3 concentration could become limiting in the α-amylase response.

Exposure of isolated *Rht* aleurone tissue from wheat to 5 C temperature for 20 h prior to the addition of exogenous GA_3, resulted in significant changes in the content of membrane phospholipids (Cleland, 1986; Singh and Paleg, 1984c, 1986b) and fatty acids (Singh and Paleg, 1986b). Pretreatment of aleurone tissue with exogenous IAA application also increased the content of fatty acids in the cell membrane. Changes in lipid metabolism are among the earliest effects caused by GA_3 in barley aleurone cells (Fernandez and Staehelin, 1987). These results suggest the possibility that the GA_3 receptor sites are membrane-based lipids (Singh and Paleg, 1984c). The increase in total phospholipids, coincided exactly on a temporal basis with induced GA_3 sensitivity which was induced by exposure to the same low temperatures (Singh and Paleg, 1986b). In conclusion, results indicate that the basis of the GA_3 insensitivity of the *Rht* allele resides in an aberrant phospholipid/fatty acid composition and/or metabolism;

furthermore, exposure to 5 C for 20 h or longer, or 342 nM IAA for 4 h or longer reverses or corrects the genetic deficiency, enabling the tissue to adopt a GA_3 responsive membrane composition (Singh and Paleg, 1986b). Lipase can be transferred from protein bodies (storage form) to lipid bodies (active form) by lateral diffusion within the plane of the fused phospholipid monolayer, and that transfer can be controlled by GA through an unknown mechanism (Fernandez and Staehelin, 1987).

Finally, Gale and Youssefian (1985) hypothesized that an auxin might be involved in the hormonal lesion of the dwarf genotypes. Using maize coleoptiles, Hager et al. (1991) reported an auxin-enhanced membrane flow from the endoplasmic reticulum to the plasma membrane. The center of auxin production in the *Avena* seedling resides in the endosperm initially and also within half an hour after soaking. Later, the auxin production was transferred to the *Avena* coleoptile tip when the coleoptile was no more than 1.5 mm in length. The maximal auxin production was reached when the coleoptile was about 10 mm long (Thimann and O'Brien, 1965). The timing for wheat has not been studied. The apical millimeter or so of the coleoptile synthesized IAA which was transported to the growing coleoptile regions below the tip (O'Brien and Thimann, 1965). Auxin causes elongation growth of plant cells by increasing the plastic extensibility of the cell wall (Hager et al., 1991). Kaufman et al. (1968) showed that in *Avena* internodes acid-induced growth is observable even though the tissue is unresponsive to auxin. Pretreatment (0.5-8 h) with GA_3 enhanced the subsequent response of cucumber hypocotyl segments to auxin (Zeroni and Hall, 1980).

Dwarf Seedlings and Coleoptile Studies

The coleoptile seems to perform two major functions in nature: it guides the shoot to the soil surface through its great sensitivity to light and gravity, and it protects the shoot en route (O'Brien and Thimann, 1965). *Rht* alleles reduce the elongation of coleoptiles, leaves and stems (Lenton et al., 1987). In *Avena* coleoptiles, cell elongation is by far the dominant component of growth (Kaufman et al., 1965). Coleoptiles have been studied for many years, although the main emphasis has been placed on their physiological properties (Parodi et al., 1970). One of the physiological aspects studied on seedlings and coleoptiles is the GA-insensitivity (Allan et al., 1961). GA-insensitivity is detected by treating seedlings with low concentrations of exogenous GA. Varieties lacking GA insensitivity, respond by elongating their first leaf sheath (Gale and Marshall, 1975). There is some indication that dwarf seedlings are stimulated by GA_3 at low temperatures (Miralles and Slafer, 1995). On the other hand, there are no reports on the coleoptile length of dwarf seedlings being stimulated by GA_3 at normal growing temperatures (Allan et al., 1961; Keyes et al., 1989) or at low temperatures (Pinthus and Abraham, 1996). Studies on wheat coleoptiles reported that when seeds of *rht* and *Rht* genotypes were sown on vermiculite soaked with GA_3 (25 ppm) at 11 and 25 C, coleoptiles of *Rht* seeds did not respond to GA_3 application at either temperature. These findings at the coleoptile level, do not support the results obtained by several other researchers on the successful effect of low temperatures in inducing GA_3 sensitivity in aleurone and mature tissues of *Rht* genotypes. For this reason, a more complete and detailed study in the effect of low temperature and GA_3 application on coleoptile length of *Rht* seeds should be conducted.

CHAPTER 3
THE EFFECT OF GIBBERELLIC ACID AND TEMPERATURE ON SEEDLING CHARACTERISTICS OF DWARF ISOLINES

Introduction

Gibberellic acid (GA) is one of the most important growth regulators/plant hormones. GA has several functions in plants, beginning in the seed germination phase by promoting α-amylase production for endosperm starch hydrolysis and extending throughout all the processes of plant growth and development (Taneyama et al., 1995; Großelindemann et al., 1991; Radley, 1970). GA is known as the hormone that promotes cell elongation in different plant organs and tissues. The first leaf of dwarf wheat isolines were reported in the literature not to respond to exogenous GA application (Borner, 1991; Gale and Marshall, 1973, 1975, 1976; Singh and Paleg, 1984a, 1986a, 1986b; Youssefian et al., 1992a, 1992b), although reports on the effect of exogenous GA application on the coleoptile are extremely rare (Allan et al., 1961; Pinthus and Abraham, 1996).

The purpose of this study was to determine the combined effect of temperature, genetic background, isoline and GA concentrations on various seedling characters (coleoptile, first leaf, root length) when applied in the germination medium.

Materials and Methods

All combinations of four isolines within each of two (Marfed-spring cultivar, Burt-winter cultivar) genetic backgrounds (GB) produced in Pullman-Washington in 1989 in the field and stored after harvest at -20 C were used. The phenotype and genotypes of the four isolines within each of the two genetic backgrounds were: normal height or tall [(T-0) ($rht_1rht_1rht_2rht_2$)], semidwarf-1 [(SD-1) ($Rht_1Rht_1rht_2rht_2$)], semidwarf-2 [(SD-2) ($rht_1rht_1Rht_2Rht_2$)], and dwarf [(D-12) ($Rht_1Rht_1Rht_2Rht_2$)].

Seeds of each isoline-genetic background combination were soaked for 4 days at 2 C in 10 ml of five GA concentrations (0, 5, 50, 100, 500 mg L^{-1}) plus 0.2 ml L^{-1} Vitavax ® 200 (Gustafson, McKinney, TX 75070), fungicide for fungi control and then enfolded in germination paper inserted in 16.5 x 17.8 cm plastic growth pouches (Mega International of Minneapolis, Minneapolis, MN 55416) containing 25 ml of the same GA solution used for soaking. The seeds were oriented in the germination paper with their embryos down to ensure straight coleoptile, shoot and root growth. The pouches were placed in vertical partitions of closed transparent plexiglass containers with 100% relative humidity during the germination process. Each treatment-combination was replicated within and on each of two dates. The containers were placed randomly inside a constant temperature chamber in the dark for 9, 26 and 68 days at 18, 10, and 2 C, respectively, (Reid et al., 1974). The number of days at each temperature was selected to assure that the first leaf would have emerged through the tip of the coleoptile in all seedlings. At the end of each period, the coleoptile, first leaf, and root length of 20 seedlings in each replication were measured in millimeters (mm), making a total of 40 seedlings for each treatment combination. The coleoptiles were measured after the first leaf had emerged from the coleoptile tip (Allan et al., 1965) in the seedlings measured. The first leaf length was measured from the coleoptile tip to the leaf tip. This can also be called measurement of the leaf sheath.

A complete factorial variance analysis involving all combinations of the main effects, GA concentration, genetic background, isoline, temperature and date, was performed (SAS Inst., 1988). The minimum differences for significance presented in the tables were obtained using the Duncan's range values for the maximum number of means to be compared (Harter, 1960).

Results

Coleoptile Length (CL)

The analysis of variance is presented in Table 3-1. The main effect GA concentration, I, GB and T and all interactions were highly significant.

The means are presented in Table 3-2. With decreasing temperatures, CL increased in magnitude and significance. On the other hand, with increasing GA concentrations, CL increased significantly. Marfed was significantly longer than Burt regardless of temperature or GA concentration, except at 10 C where the GB length (over isolines) at 0 mg L^{-1} GA were not different. In general, and regardless of the T, GB and GA concentration, T-0 always had the longest CL followed by SD-1, SD-2 and D-12. However, those differences were more pronounced in Burt than in Marfed.

The means representing the IxGA interaction are shown in Fig. 3-1. The effect of 5 mg L^{-1} GA on promoting CL was quite pronounced at 2 and 10 less so at 18 C. Similar trends were observed at 500 mg L^{-1} GA and the effect was more obvious at the lower temperatures.

Table 3-1. Mean squares from the analysis of variance on the effect of gibberellic acid (GA), four dwarf isolines in two genetic backgrounds and three temperatures, on the coleoptile, first leaf and root length of seedlings grown in the dark.

Source of variation	df	Length		
		Coleoptile	First leaf	Root
GA concentration (GA)	4	2280**	307776**	164427**
Isoline (I)	3	213221**	282204**	46469**
GAxI	12	2780**	29318**	3547**
Genetic background (GB)	1	100001**	127741**	99**
GBxGA	4	4552**	5237**	2191**
GBxI	3	5780**	71	1556**
GBxGAxI	12	4796**	2308**	1351**
Temperature (T)	2	904775**	68066**	3854674**
TxGA	8	2947**	2454**	28019**
TxI	6	5794**	32614**	9889**
TxGB	2	8241**	6007**	2509**
TxGAxI	24	350**	3503**	1814**
TxGAxGB	8	734**	2599**	2029**
TxIxGB	6	980**	9999**	2719**
TxIxGBxGA	24	143**	1248**	759**
Date (D)	1	1440**	56129**	98364**
DxGA	4	436**	3541**	17900**
DxI	3	1338**	3041**	359
DxGAxI	12	96	761	977**
DxGB	1	16144**	12109**	13012**
DxGBxGA	4	67	618	2630**
DxGBxI	3	69	751	1889**
DxGBxGAxI	12	62	435	892**
DxT	2	15405**	11054**	52183**
DxTxGA	8	521**	5822**	12233**
DxTxI	6	2466**	9875**	790**
DxTxGAxI	24	87	1555**	1050**
DxTxGB	2	9001**	7235**	689
DxTxGBxGA	8	84	3086**	3054**
DxTxGBxI	6	230**	452	1298**
DxTxGBxGAxI	24	91	1020	903**
Error	4560	79	714	263

**F value significant at the 1% level.

Table 3-2. Coleoptile length means showing the effect of five gibberellic acid concentrations on four dwarf wheat isolines [normal height or tall (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in two genetic backgrounds (Marfed, Burt) and three (2, 10, 18 C) temperatures (T), germinated in the dark.

T	Genetic background	Isoline	GA concentration (mg L^{-1})				
			0	5	50	100	500
			mm				
2 C	Marfed	T-0	105†	135	132	132	148
		SD-1	112	125	127	119	136
		SD-2	99	115	117	110	125
		D-12	100	102	105	101	112
	Burt	T-0	100	121	119	108	124
		SD-1	100	113	107	102	108
		SD-2	97	106	107	98	108
		D-12	91	99	95	92	95
10 C	Marfed	T-0	82	114	118	121	124
		SD-1	83	92	101	97	102
		SD-2	70	81	84	86	86
		D-12	63	66	66	69	72
	Burt	T-0	88	101	99	100	104
		SD-1	74	75	77	78	82
		SD-2	75	79	77	75	79
		D-12	63	63	64	64	64
18 C	Marfed	T-0	75	80	81	84	96
		SD-1	62	68	70	70	72
		SD-2	58	58	58	60	63
		D-12	51	51	52	53	53
	Burt	T-0	77	81	81	82	89
		SD-1	56	59	60	60	62
		SD-2	56	56	57	57	57
		D-12	48	48	48	48	48

† Minimum differences among any combination of temperature-genetic background-isoline-GA concentration means were 5 and 7 for 5 and 1%, respectively.

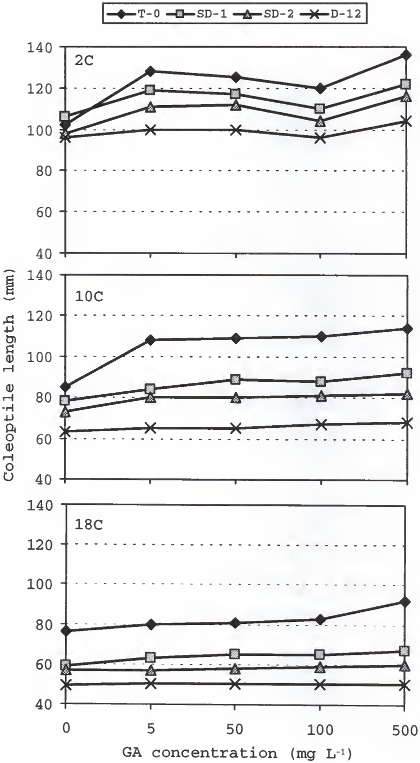

Fig. 3-1. The effect of five gibberellic acid (GA) concentrations on the coleoptile length of four dwarf wheat isolines, over two genetic backgrounds, germinated in the dark at 2, 10 and 18C.

First Leaf Length (FLL)

The analysis of variance is presented in Table 3-1. The main effect GA concentration, I, GB and T and all interactions except GBxI were highly significant.

The means are presented in Table 3-3. FLL comparisons among temperatures was not possible since the seedlings were not at the same physiological stage at each reading period in each temperature. The number of days in each experiment temperature was selected to assure that the first leaf would have emerged through the tip of the coleoptile in all seedlings. In other words, the coleoptile would have reached its maximum length. The equivalent number of physiological days to grow leaves at different temperatures was not determined. Our comparisons are limited within T, and mainly among the responses obtained at I and GB, in each GA concentration. The FLL increased significantly with increasing GA concentrations. Values obtained by subtracting the FLL of the control treatment from the FLL at 500 mg L^{-1} GA showed that the FLL increases in the three dwarf isolines were more pronounced at 2 and 10 C and less at 18 C. On the other hand, T-0 increases were more pronounced at higher temperatures. In general, Marfed and Burt were not different. T-0 had the longest FLL both SD-1 and SD-2 not different from each other and D-12 the shortest.

Table 3-3. First leaf length means showing the effect of five gibberellic acid concentrations on four dwarf wheat isolines [normal height or tall (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in two genetic backgrounds (Marfed, Burt) and three (2, 10, 18 C) temperatures (T), germinated in the dark.

			GA concentration (mg L^{-1})				
T	Genetic background	Isoline	0	5	50	100	500
			mm				
2 C	Marfed	T-0	26†	50	47	51	82
		SD-1	27	50	45	65	59
		SD-2	51	57	62	59	85
		D-12	18	36	38	31	54
	Burt	T-0	39	60	83	88	120
		SD-1	31	44	58	64	82
		SD-2	36	58	71	87	85
		D-12	25	43	45	51	49
10 C	Marfed	T-0	44	77	85	85	116
		SD-1	39	46	60	56	86
		SD-2	39	45	60	61	86
		D-12	40	38	44	45	62
	Burt	T-0	40	70	85	96	143
		SD-1	40	58	71	72	86
		SD-2	41	58	71	71	87
		D-12	45	49	56	60	66
18 C	Marfed	T-0	44	81	108	119	157
		SD-1	43	48	57	59	71
		SD-2	38	44	49	53	64
		D-12	34	37	41	42	50
	Burt	T-0	48	73	90	127	166
		SD-1	60	56	65	73	98
		SD-2	58	60	64	75	89
		D-12	53	52	55	55	69

† Minimum differences among any combination of temperature-genetic background-isoline-GA concentration means were 16 and 20 for 5 and 1%, respectively.

The means representing the IxGA interactions are shown in Fig. 3-2. The effect of GA in promoting the length was quite pronounced in all temperatures with the effect especially pronounced in T-0 at 500 mg L^{-1}.

Root Length (RL)

The analysis of variance is presented in Table 3-1. The main effects, GA concentration, I, GB and T and all their interactions were highly significant.

The means are presented in Table 3-4. RL decreased sharply with increasing GA concentration and decreasing temperature. The extreme reduction at lower temperatures especially at higher GA concentrations would likely impair function. The RL of Marfed was somewhat longer than Burt in all T and GA concentrations. In general, over GA concentrations and GB, T-0 had the shortest and D-12 the longest roots at all temperatures. However, the differences were more pronounced at 18 C and less so, at 2 C.

The means representing IxGA interactions are shown in Fig. 3-3. With an increase in GA concentration from 0 to 50 mg L^{-1} GA, the RL decrease was quite pronounced. On the other hand, 100 mg L^{-1} GA slightly increased RL of all isolines at 10 and 18 C. At 2 C, that effect was almost non-existent.

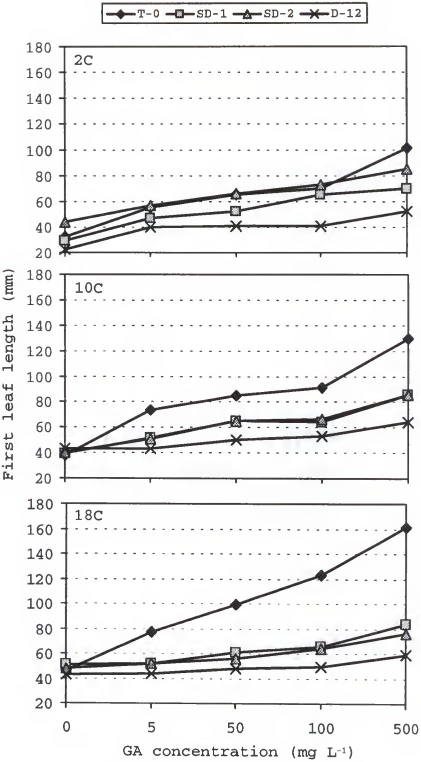

Fig. 3-2. The effect of five gibberellic acid (GA) concentrations on the first leaf length of four dwarf wheat isolines, over two genetic backgrounds, germinated in the dark at 2, 10 and 18C.

Table 3-4. Root length means showing the effect of five gibberellic acid concentrations on four dwarf wheat isolines [normal height or tall (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in two genetic backgrounds (Marfed, Burt) and three (2, 10, 18 C) temperatures (T), germinated in the dark.

			GA concentration (mg L^{-1})				
T	Genetic background	Isoline	0	5	50	100	500
			mm				
2 C	Marfed	T-0	112[†]	80	68	64	61
		SD-1	93	78	78	68	71
		SD-2	102	80	84	61	72
		D-12	88	76	82	73	69
	Burt	T-0	100	66	48	50	56
		SD-1	103	77	72	66	67
		SD-2	95	74	68	55	56
		D-12	96	80	79	74	73
10 C	Marfed	T-0	138	117	110	110	115
		SD-1	134	124	125	117	121
		SD-2	134	126	122	134	121
		D-12	133	126	125	134	116
	Burt	T-0	120	111	115	111	105
		SD-1	119	133	117	122	105
		SD-2	122	134	126	120	103
		D-12	118	124	116	121	106
18 C	Marfed	T-0	189	159	152	144	133
		SD-1	205	182	170	183	151
		SD-2	207	196	166	185	152
		D-12	207	195	174	188	149
	Burt	T-0	188	159	143	161	132
		SD-1	197	184	171	190	146
		SD-2	201	196	163	182	141
		D-12	210	199	173	167	147

[†] Minimum differences among any combination of temperature-genetic background-isoline-GA concentration means were 9 and 12 for 5 and 1%, respectively.

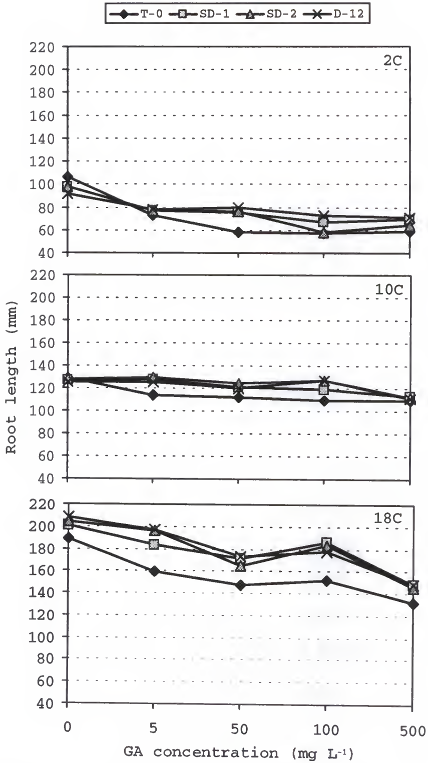

Fig. 3-3. The effect of five gibberellic acid (GA) concentrations on the root length of four dwarf wheat isolines, over two genetic backgrounds, germinated in the dark at 2, 10 and 18C.

Discussion

The results of this study indicated that GA applied in the germination medium and temperature altered coleoptile, first leaf, and root length of dwarf wheat isolines. Overall, coleoptiles, first leaves and roots became more sensitive to GA application as the temperature was decreased. Coleoptile and first leaf sensitivity to GA was expressed as increased elongation, while an inhibition of growth was observed in roots. Previous studies reported no coleoptile differences resulting from GA treatment (Allan et al., 1961). The effect of temperature on the coleoptile length was so pronounced that seedlings growing at 10 C with 500 mg L^{-1} GA had shorter coleoptiles than those growing at 2 C with no GA. Those differences were not as pronounced at 18 C, although coleoptiles of seedlings growing at 10 C were always longer than those growing at 18 C. Basically, higher temperatures are known to inhibit coleoptile cell elongation (Allan et al., 1962). Although coleoptile elongation above the control occurred at all GA concentrations, responses in descending order were higher in T-0 (no dominant dwarf allele), intermediate in SD-1 and SD-2 (one dominant dwarfing allele), and almost non-existent in D-12 (two dominant dwarfing alleles). As the temperature increased, higher GA concentrations were necessary to obtain increases in coleoptile length above the control, indicating that GA insensitivity in dwarf isolines might be caused by a defective hormone receptor (Rodaway et al., 1991) expressed at higher temperatures. These receptors are usually localized in the cell wall or cell membrane (Trewavas, 1982). It is not known if low temperatures are able to modify the hormone receptor site or allow GA to enter the cell by bypassing the hormone receptor. The fact is that dwarf wheat expresses sensitivity to exogenously-applied GA at lower temperatures. It was suggested that lower temperatures might also induce an increase in the hormone receptor sites (Singh and Paleg, 1984a; 1984b; 1984c). These results showed that after the initial coleoptile elongation from an application of 5 mg GA L^{-1}, the means of the four dwarf wheat isolines did not change statistically as the GA concentration increased. When 500 mg L^{-1} GA was applied however, T-0 and SD-1 elongated to a greater extent. This is an indication that the combination of 2 C and 5 mg L^{-1} GA, was probably the ideal combination for maximum coleoptile elongation in the known 'GA insensitive' dwarf wheat. The coleoptile of the GA sensitive isoline (T-0) did not elongate further with increasing GA concentrations indicating that the coleoptile cells had reached their maximum potential of elongation (cell wall stretching). When talls and dwarfs (sensitive and insensitive genotypes to GA) responded to the application of GA in a similar pattern, this indicated that the dwarfing allele(s) is not the cause of insensitivity in those particular environments.

Genetic background played an important role in the response. Marfed was more sensitive to GA than Burt, independent of temperature. This observation cannot be easily correlated to the winter/spring growth habit. Both GB performed similarly in all temperatures. Apparently, other genetic factors besides the dominant dwarf alleles may be contributing to the response of these genotypes to exogenous GA application. Nevertheless, the sensitivity/insensitivity issue is largely controlled by the dominant dwarf allele(s).

Interactions between temperature and GA application on the first leaf suggests that the 'sensitivity' observed was influenced by the cold temperatures. However, in a pattern different from the one observed with coleoptiles, GA application resulted in all leaves elongating significantly in all three temperatures. The first leaf did not reach maximum elongation with 500 mg L^{-1} GA. In fact, GA might be more effective on promoting first leaf elongation than temperature. The longest leaves at 0 mg L^{-1} GA concentration were obtained at higher temperatures. The 500 mg L^{-1} GA application resulted in maximum first leaf lengths in all isolines. This was not the case with coleoptiles. Two reasons can be given to explain these differences. While the lower temperature stimulated cell elongation in the coleoptiles, there might be a type of inhibition with the first leaves. Or, it can be that, because high temperatures and coleoptile lengths are negatively related, the first leaf emerges from the coleoptile earlier and grows a longer period of time. At lower temperatures, however, the coleoptile grows longer with the first leaf enclosed. Obviously, a different mechanism exists for controlling coleoptile and leaf elongation as far as the effect of temperature and GA interactions are concerned.

The effect of genetic background on the first leaf and its interactions with GA and temperature also differed from their effect on coleoptiles. For example, at 2 C and 50 mg L^{-1} GA, all Burt isolines increased their first leaf length while in Marfed, 500 mg L^{-1} GA was needed to produce that effect. At 10 C, 5 mg L^{-1} GA increased T-0, SD-1 and SD-2 of Burt, while, in Marfed, 50 mg L^{-1} GA were required to obtain the same type response. At 18 C, Marfed SD-1 and SD-2 elongated with 50 mg L^{-1} while the same Burt isolines required 100 mg L^{-1} GA. These different GB responses did not follow any specific pattern. In conclusion, the first leaf of the winter GB (Burt) seems to be more responsive to GA (responds at lower GA concentrations) when grown at cooler temperatures. On the other hand, the spring GB (Marfed) might be more responsive to GA when growing at higher temperatures. Most studies report the first leaf of dwarf wheat isolines as being GA insensitive (Appleford and Lenton, 1991; Baroncelli et al., 1988; Keyes et al., 1990). Those experiments were conducted at 20-25 C with GA concentrations of 5-50 mg L^{-1}. If, in this study, temperatures above 18 C would have been tested, most probably all the significance and interactions would not have been present.

Roots were negatively affected by both GA and temperature. Similarly, Sullivan and Pfahler (1985) found that 12 C decreased root growth in rye. Averaged across both GB, 2 C and 5 mg L^{-1} GA caused a drastic decrease in the root length in all isolines. Overall, a small GA concentration caused dwarf wheat root length to decrease, regardless of temperature. GA applications have been reported to cause lower root dry weights (Gale and Marshall, 1973). Obviously, GA caused the inverse effect on roots than on coleoptiles and leaves. The latter two have the tendency to elongate in response to GA, while, the effect on roots is inhibition. Roots by their nature are different from leaves and coleoptiles. They grow subterraneanly and geotropically. In seedling studies, the reserves for growth are limited by the amount of reserves in the seed. Reasons for the negative GA effect on root growth could be postulated. Either the seed reserves are dispensed to the coleoptile and first leaf growth, leaving the roots deprived of nutrients and consequently smaller in total length, or, a direct effect of GA on root growth causes inhibition. Root growth is more associated with cell division rather than cell extension/elongation as in coleoptiles, leaves and stems. It might be that GA causes an inhibition of root cell division.

The observed interactions between GA and root length are also temperature and isoline dependent. In the field, it was reported that root growth may exceed shoot growth at low temperatures, but as temperatures increased the growth of shoots increased more than that of roots (Evans et al., 1995). This might explain why, at 10 C, GA did not affect the root length in any isoline or GB, however, at 18 C, the roots are more sensitive to temperature and GA.

The highly significant interaction between temperature, isoline, and GA concentration suggests that the reported coleoptile and first leaf GA-insensitivity in dwarf wheat can be partially overcome by the combination of low temperatures and high GA concentrations. Apparently, the physiological action of the dominant dwarfing alleles is capable of modification by environmental and genetic factors. This study shows that the exogenous application of growth regulators as a seed treatment may be effective in at least partially overcoming the reduced coleoptile length associated with dwarfing in wheat.

CHAPTER 4
THE EFFECT OF VARIOUS INORGANIC AND ORGANIC COMPOUNDS ON SEEDLING CHARACTERISTICS OF DWARF ISOLINES

Introduction

Gibberellic acid (GA), a natural plant growth regulator, has been reported to overcome genetic dwarfing in many plant species (Phinney, 1984). Many studies (Appleford and Lenton, 1991; Beharav et al., 1994; Borner, 1991; Gale and Marshall, 1973, 1975, 1976; Lenton et al., 1987; Singh and Paleg, 1984a, 1986a, 1986b; Youssefian et al., 1992a, 1992b;) with dwarfing in wheat have concluded that dwarfing in wheat are 'GA-insensitive.' A number of organic and inorganic compounds which have been reported to influence germination rate, seedling growth, and development, have not been tested to determine their effect on overcoming dwarfing in wheat (Bewley and Black, 1978; 1984).

The objective of this study was to evaluate the effect of various compounds and their combinations in the germination medium on coleoptile elongation and other seedling characters in dwarf isolines.

Materials and Methods

Seeds of four isolines of Marfed (spring wheat genetic background) which were produced in Gainesville, Florida, in 1996 and were stored at -20 C immediately after harvest, were tested. The name, abbreviation and genotype of these four homozygous and homogenous isolines are respectively as follows: normal height or tall, T-0, $rht_1rht_1rht_2rht_2$; semidwarf-1, SD-1, $Rht_1Rht_1rht_2rht_2$; semidwarf-2, SD-2, $rht_1rht_1Rht_2Rht_2$; and dwarf, D-12, $Rht_1Rht_1Rht_2Rht_2$.

The concentration of each compound in each of the twenty solutions used as the soaking and germination medium are presented in Table 4-1. Seeds of each isoline were soaked in 10 ml for 2 days at 5 C in each of the twenty solutions plus 0.2 ml L^{-1} Vitavax ® 200 (Gustafson, McKinney, TX 75070), fungicide for fungi control and then enfolded in germination paper inserted in 16.5 x 17.8 cm plastic growth pouches (Mega International of Minneapolis, Minneapolis, MN 55416) containing 25 ml of the same solution used for soaking. The seeds were oriented in the germination paper with their embryos down to ensure straight coleoptile, shoot and root growth. Three pouches (10 seeds each) were used for each treatment isoline-solution combination. The pouches were placed in vertical partitions of closed transparent plexiglass containers with 100% relative humidity during the germination process.

Table 4-1. Name, abbreviation and compound formula and concentration of each of the twenty solutions used as soaking and germination medium of seeds from four dwarf wheat isolines. The coleoptile, first leaf and root length of seedlings germinated in the dark at 18 C were measured.

Solution	Concentration and formula of each compound
Water	deionized water (H_2O)
GA	500 mg gibberellic acid A_3 ($C_{19}H_{21}O_6K$) L^{-1} H_2O (500 ppm GA)
Ca	100 mg calcium nitrate [$Ca(NO_3)_2{\bullet}4H_2O$] L^{-1} H_2O (100 ppm Ca)
GL	10 g glucose ($C_6H_{12}O_6$) L^{-1} H_2O (10^4 ppm GL)
PEG	96.3 g polyethylene glycol 8000 L^{-1} H_2O† (PEG)
24D	1 mg dimethylamine salt of 2,4-dichlorophenoxyacetic acid L^{-1} H_2O (1 ppm 2,4-D)
NAA	10 mg ß-naphthaleneacetic acid ($C_{12}H_{10}O_2$) L^{-1} H_2O (10 ppm NAA)
GA+Ca	500 ppm GA + 100 ppm Ca
GA+GL	500 ppm GA + 10^4 ppm GL
GA+PEG	500 ppm GA + PEG
GA+24D	500 ppm GA + 1 ppm 2,4-D
GA+NAA	500 ppm GA + 10 ppm NAA
GA+Ca+GL	500 ppm GA + 100 ppm Ca + 10^4 ppm GL
GA+Ca+PEG	500 ppm GA + 100 ppm Ca + PEG
GA+Ca+24D	500 ppm GA + 100 ppm Ca + 1 ppm 2,4-D
GA+Ca+NAA	500 ppm GA + 100 ppm Ca + 10 ppm NAA
GA+24D+NAA	500 ppm GA + 1 ppm 2,4-D + 10 ppm NAA
GA+GL+PEG	500 ppm GA + 10^4 ppm GL + PEG
GA+GL+24D	500 ppm GA + 10^4 ppm GL + 1 ppm 2,4-D
GA+GL+NAA	500 ppm GA + 10^4 ppm GL + 10 ppm NAA

† -0.156 Mpa osmotic potential (Michel and Kaufman, 1973)

Each container had 20 partitions which held the pouches vertically. The containers were placed randomly inside a constant temperature chamber at 18 C in the dark. The coleoptile, first leaf, and root length of 15 seedlings were measured in millimeters (mm) at 9 days after the first leaf had emerged through the coleoptile tip in the seedlings. The experiment was repeated on each of two dates making a total of 30 seedlings for each treatment combination.

A complete factorial variance analysis involving all combinations of the main effects, solution, isoline and date, was performed on each character (SAS Inst., 1988). The minimum differences for significance presented in the tables were obtained using the Duncan's multiple range values for the maximum number of means to be compared (Harter, 1960).

Results

Coleoptile Length

The analysis of variance is presented in Table 4-2. All main effects and their interaction were significant at the 1% level.

The means are presented in Table 4-3. In water, the means of T-0, SD-1, SD-2 and D-12 were 100, 77, 62 and 58 mm, respectively. Compared to water, GA increased T-0 by 18 mm, SD-1 by 8 mm, SD-2 by 5 mm and D-12 by 4 mm. In general, the response of the four isolines to the 19 solutions

Table 4-2. Mean squares from the analysis of variance on the effect of twenty solutions on four dwarf wheat isolines, two dates, on the coleoptile, first leaf and root length of seedlings germinated in the dark at 18 C on two dates.

Source of variation	df	Length Coleoptile	 First leaf	 Root
Solutions (S)	19	3604**	103599**	249238**
Isoline (I)	3	50464**	482766**	4399**
SxI	57	549**	20643**	709**
Date (D)	1	18432**	96850**	1915**
DxS	19	538**	3980**	1357**
DxI	3	310**	14908**	375
DxSxI	57	81**	605	786**
Error	2240	43	477	306

**F value significant at the 5% level.

Table 4-3. Coleoptile length means showing the effect of twenty solutions on four dwarf wheat isolines [normal height or tall (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] germinated in the dark at 18 C on two dates.

	Isoline			
Solution	T-0	SD-1	SD-2	D-12
Water	100†	77	62	58
GA	118	85	67	62
Ca	100	77	64	58
GL	103	79	62	59
PEG	115	86	70	63
24D	104	83	68	63
NAA	109	80	67	62
GA+Ca	118	85	69	63
GA+GL	129	88	70	63
GA+PEG	130	91	73	65
GA+24D	122	90	73	64
GA+NAA	122	87	72	64
GA+Ca+GL	117	85	65	62
GA+Ca+PEG	132	92	73	64
GA+Ca+24D	124	89	73	62
GA+Ca+NAA	117	89	72	65
GA+24D+NAA	118	87	72	63
GA+GL+PEG	127	89	76	65
GA+GL+24D	94	74	64	55
GA+GL+NAA	127	86	69	59

† Minimum differences among any combination of isoline-solution means were 4 and 6 at the 5 and 1% level, respectively.

followed the same pattern with T-0 showing the largest response and D-12, little or no change.

The effect of the solutions containing Ca, GL, PEG, 24D and NAA singly was intermediate in all isolines with none significantly outside the water - GA range. However, when GA was included in the solution with other compounds, synergistic effects over GA alone were observed, especially in T-0 and SD-1. For example, the means of GA+Ca+PEG for T-0, SD-1 and SD-2, were significantly higher than either water or GA.

The length changes among isolines in selected solutions in which significant differences were present, are shown in Fig. 4-1. PEG applied to the germination medium increased the isoline means to the same level as GA. On the other hand, the isoline coleoptile means (except D-12) were significantly longer in GA+Ca+PEG than the respective means in GA. Of the four isolines, only T-0 and SD-2 were significantly increased above GA by GA+GL.

First Leaf Length

The analysis of variance is presented in Table 4-2. The main effects, S and I, and their interaction were significant at the 1% level.

The means are presented in Table 4-4. In water, the means of T-0, SD-1, SD-2 and D-12 were 75, 50, 58 and 38 mm, respectively. Compared to water, GA increased T-0 by 162 mm,

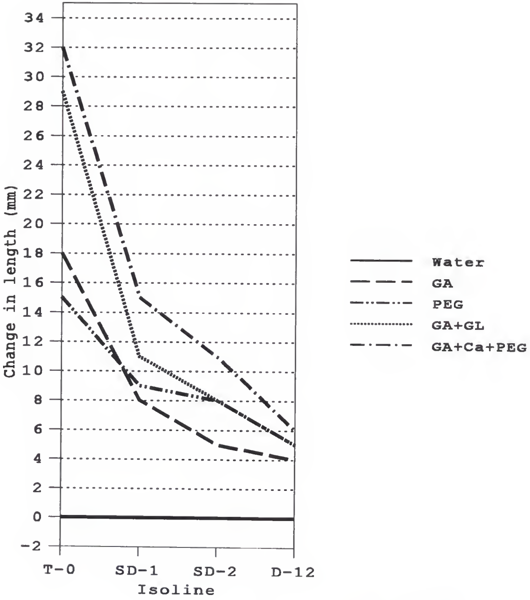

Fig. 4-1. Change (mean of water - mean of solution of interest) in coleoptile length of each isoline by selected solutions compared to water.

Table 4-4. First leaf length means showing the effect of twenty solutions on four dwarf wheat isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] germinated in the dark at 18 C on two dates.

	Isoline			
Solution	T-0	SD-1	SD-2	D-12
Water	75†	50	58	38
GA	237	91	90	47
Ca	50	46	54	40
GL	22	29	36	18
PEG	23	31	36	25
24D	54	39	47	27
NAA	95	52	61	31
GA+Ca	208	98	102	49
GA+GL	115	58	69	29
GA+PEG	183	61	61	31
GA+24D	84	50	55	24
GA+NAA	185	73	79	37
GA+Ca+GL	92	40	58	21
GA+Ca+PEG	149	76	86	40
GA+Ca+24D	83	46	52	27
GA+Ca+NAA	134	61	63	33
GA+24D+NAA	26	20	28	15
GA+GL+PEG	23	19	33	12
GA+GL+24D	17	14	26	9
GA+GL+NAA	74	38	55	19

† Minimum differences among any combination of isoline-solution means were 14 and 19 at the 5 and 1% level, respectively.

SD-1 by 41 mm, SD-2 by 32 mm and D-12 by 9 mm. In general, the response of the four isolines to the 19 solutions followed the same pattern with T-0 showing the largest response and D-12, a considerably smaller change.

The solutions containing Ca, GL, PEG and 24D singly sharply reduced the means below water with the change less pronounced with NAA. The effects of the interactions among various compounds when combined were very unpredictable. In general, all of the isoline means in the 18 solutions were either significantly less or equal to the GA mean.

The length changes among isolines in selected solutions in which significant differences were present are shown in Fig. 4-2. GA resulted in significant increases in all isolines except in D-12. Ca and NAA had no effect on any isoline, except T-0, which had a significant increase with NAA. PEG, however, severely reduced first leaf length means.

Root Length

The analysis of variance is presented in Table 4-2. The main effects, S and I, and their interaction were significant at the 1% level.

The means are presented in Table 4-5. In water, the means of T-0, SD-1, SD-2 and D-12 were 137, 144, 136 and 130 mm, respectively. Compared to water, GA decreased the means slightly and by approximately the same amount. In general,

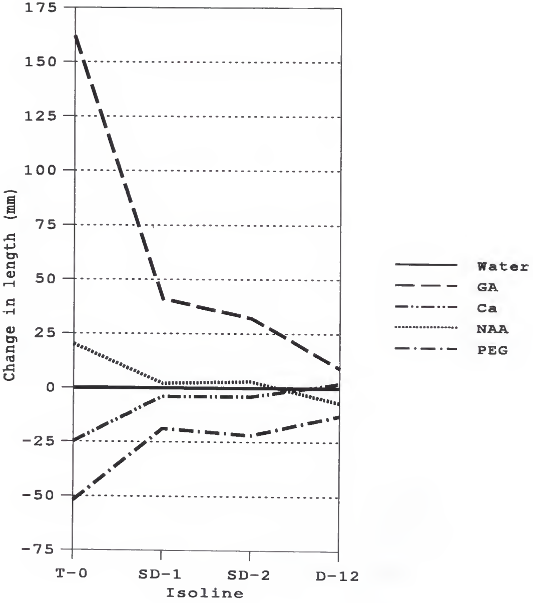

Fig. 4-2. Change (mean of water - mean of solution of interest) in first leaf length of each isoline by selected solutions compared to water.

Table 4-5. Root length means showing the effect of twenty solutions on four dwarf wheat isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] germinated in the dark at 18 C on two dates.

	Isoline			
Solution	T-0	SD-1	SD-2	D-12
Water	137†	144	136	130
GA	126	132	131	125
Ca	146	148	140	142
GL	123	128	121	114
PEG	154	156	148	150
24D	57	52	49	48
NAA	144	152	161	146
GA+Ca	139	145	142	137
GA+GL	115	118	116	113
GA+PEG	142	148	134	132
GA+24D	46	53	45	43
GA+NAA	122	135	144	135
GA+Ca+GL	75	84	66	71
GA+Ca+PEG	155	157	155	150
GA+Ca+24D	33	41	45	32
GA+Ca+NAA	121	126	105	118
GA+24D+NAA	27	23	22	20
GA+GL+PEG	118	125	126	123
GA+GL+24D	22	21	22	21
GA+GL+NAA	101	85	92	91

† Minimum differences among any combination of isoline-solution means were 11 and 15 at the 5 and 1% level, respectively.

most solutions decreased means, but PEG and GA+Ca+PEG produced a significant increase in all isolines. 24D and all its combinations severely reduced means in all isolines however, NAA increased the mean in all isolines.

The length changes among isolines in selected solutions in which significant differences were present, are shown in Fig. 4-3. Compared to water, GA reduced means especially in T-0 and SD-1 while NAA increased means. In all isolines, 24D severely reduced means while PEG significantly increased them compared to water.

Discussion

The results of this study showed that GA, Ca, GL, PEG, 24D, NAA and their combinations had different effects on coleoptiles, first leaves and roots of dwarf wheat isolines. Ca is one of cell wall components conferring rigidity (Moll and Jones, 1981; Arif and Newman, 1993); GL (glucose) provide an essential carbon source for the growth of young seedlings and for turgor pressure maintenance (Yu et al., 1996); PEG is used in seedling studies to simulate drought conditions by decreasing the water osmotic potential (Pfahler et al., 1987b; Morgan, 1988). 24D is a well-known herbicide which causes cells in certain plant species to divide uncontrollably (Krall, 1949). NAA, a synthetic auxin, is widely used to increase root development in propagation of stem cuttings (Gianfagna, 1995). It appears that some of

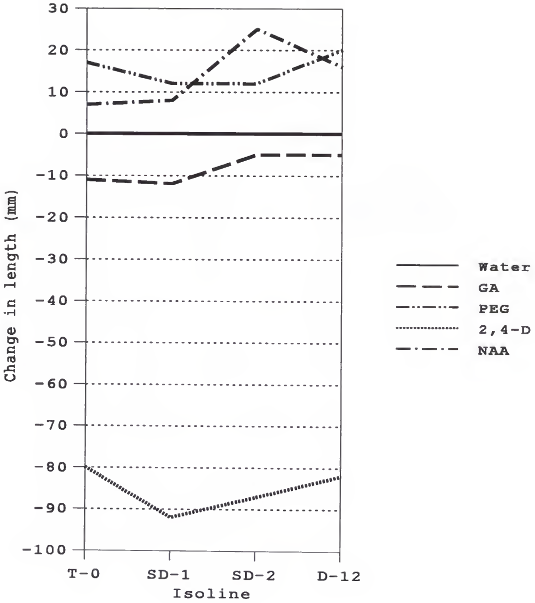

Fig. 4-2. Change (mean of water - mean of solution of interest) in root length of each isoline by selected solutions compared to water.

these compounds enhance the GA action probably by providing an energy source while softening the cell wall. Therefore, coleoptile elongation is enhanced by these combinations.

GA, in certain combinations applied in the germination medium, significantly increased coleoptile length. Those increases were of identical or greater magnitude compared to the coleoptile length increases observed with GA alone. On the other hand, most of these compounds applied to the germination medium, alone or in combination, had no effect or reduced the first leaf and root length of dwarf isolines.

The information available about the action of PEG seems to contradict the results obtained in this study. If the water potential is decreased by PEG with less water available to the seedling, one would expect coleoptiles to be shorter than in either water or GA (Michel and Kaufman, 1973). Our results showed that PEG alone promoted coleoptile elongation of the same magnitude as GA in all four isolines. This could be explained by the fact that osmotic stress increases cell wall extensibility, by increasing cell wall-bound ferulic and diferulic acids in wheat coleoptiles (Wakabayashi et al., 1997). This hypothesized reduction in the level of these two acids allows the coleoptile cells to maintain their wall extensibility. On the other hand, cell expansion may involve an increase in solutes to maintain turgor as cell volume increases (Cosgrove, 1986). Negative relationships have been found between plant tissue elongation and turgor pressure (Morgan, 1988). Basically, it is not known whether GA stimulates cell elongation by increasing turgor pressure or changing cell wall extensibility. One might attempt to explain that PEG caused cell elongation by causing a decrease in the cell turgor pressure. In pea stem tissue studies, tissue isolated from an external supply of water went through stress relaxation because of continual loosening of the cell wall (Cosgrove, 1985). PEG is a relatively inexpensive compound which probably can at least partially substitute for the use of GA in dwarf wheat.

GL used alone did not induce coleoptile elongation, whereas 24D and NAA increased length above that of water. Since GL is an energy source, it would appear that an energy shortage is not associated with reduced coleoptile growth in dwarfs. These results also indicate that 24D and NAA hold a certain capacity for cell wall elongation. Solutions containing two or more of these compounds, such as GA+PEG, GA+24D, GA+Ca+PEG, GA+Ca+24D, GA+GL+PEG promoted coleoptile length significantly above the GA control. Obviously, a synergistic effect between GA and each of the other compounds is present. The mechanisms of action and interaction of these compounds are not known. Ca used alone or in combination with GA did not cause any elongation, but GA+Ca+PEG resulted in the most pronounced synergistic effect. In other words, the longest T-0 and SD-1 coleoptiles were observed when germinated in this combination.

The NAA effect on coleoptile length was statistically significant but not biologically meaningful. NAA probably is involved in cell elongation in the coleoptile's cells. Auxins in general, provide a dramatic elongation response in isolated stems and coleoptile sections (Cleland, 1995).

GA caused the first leaf length to elongate in all isolines except D-12. Although there is a differential response between coleoptiles and first leaves for most compounds, with GA and its effect there is some predictability. Most compounds applied alone either had no effect or decreased the length of the first leaf, with the exception of NAA in T-0. In this isoline, both first leaf and coleoptile elongated significantly more than water alone in the presence of NAA. These results indicate that NAA might be stimulating cell division, thus causing a significant increase in the length or, according to Cosgrove (1985), stimulation of growth by auxin can be entirely accounted for by the change in the metabolically-maintained irreversible extensibility of the cell wall.

The effect of PEG on the first leaf was quite interesting. While causing a significant coleoptile elongation in all isolines, severe decreases in length were observed in the first leaf. The idea has been that compounds causing coleoptile elongation had similar effects on the first leaf length. This is usually the case with GA, but apparently, elongation caused by certain compounds other than GA can give differential results between the coleoptile and the first leaf.

When examining solutions consisting of more than one compound, a pattern could be discerned. For example, combinations GA+Ca, GA+NAA, GA+Ca+PEG caused an increase in the first leaf length of all isolines except D-12, compared to water alone. These solutions are the same that promoted coleoptile length. On the other hand, solutions GA+24D+NAA, GA+GL+PEG, GA+GL+24D caused a significant decrease in the first leaf length in all isolines. Although coleoptile length was not decreased below that of the water treatment by any solution, these combinations had no effect on promoting the length, except GA+GL+PEG, which significantly increased coleoptile length while decreasing first leaf length. Although GA is a component of these solutions, there was no significant leaf elongation due to its presence. It appears that 24D and the combination of GL and PEG are the compounds that inhibit the elongation of the first leaf by GA. To a certain extent, the elongation mechanism in leaves and coleoptiles is as different as the action of the compounds tested. Future seedling studies could explain the probable differences in mechanisms and different compounds should be tested separately for their effects on coleoptiles and first leaves.

In general, GA reduced root length in all isolines significantly. Identical results were obtained by (Pfahler et al., 1991a) using tall wheat genotypes. However, no compound was more damaging to the root length than 24D, used either singly or in combination. This compound which is used as a herbicide, causes unregulated cell division, but as shown here, it also causes a severe root length reduction.

A similarity between the effect of PEG in roots and coleoptiles of dwarf wheat isolines was observed. PEG, a drought simulator, used either singly or in combination increased the root length significantly. However, in the two rye cultivars studied by Pfahler et al. (1987b), the root length decreased significantly. In wheat, both roots and coleoptiles are stimulated by this organic compound, while rye is apparently inhibited by the use of PEG. It seems that, in addition to organ specificity, these compounds are also species specific. A possible explanation for the effect of PEG seen in wheat could be a type of defense mechanism against drought. If the water potential declines and water is not readily available to the seedling, roots grow longer and deeper into the soil ensuring survival of the seedling.

Glucose, singly or in combination, caused reduced root growth, but the effect was not nearly as severe as that of 24D. Readily available glucose is a source of energy and the fact that neither roots, coleoptile nor first leaves were stimulated by this compound might indicate that the dominant dwarf allele in wheat is not blocking some biochemical pathway that limits energy utilization or production.

In conclusion, the results of this study indicated that a number of compounds either singly or in combination will increase coleoptile growth, especially in T-0 and SD-1. This would suggest that possibly a preplanting seed treatment could be used to improve stands, especially in T-0 and SD-1. Since the study reported herein evaluated these compounds when applied in the germination medium, further studies testing seed treatment in a broad range of soil conditions and temperatures will be necessary.

CHAPTER 5
EFFECT OF SEED PRODUCTION SITE ON SEEDLING CHARACTERISTICS OF DWARF ISOLINES

Introduction

From the beginning of germination until exposure of the first green leaf to light, seedling growth and development is dependent on reserve carbohydrates in the endosperm, with heavier seeds generally having more energy reserves and more rapid seedling development (Bewley and Black, 1985). Low light intensity for 7-10 days after anthesis has been associated with reduced endosperm cell numbers and also final seed weight, especially at higher temperatures (Evans et al., 1975).

Many aspects of seed quality are influenced by the production site as related to parent plant vigor and seed development (Evans et al., 1995; Pfahler, 1987a). It has not been established if differences in seed weight and seedling vigor within the same genotypes, can be related to the site of production and, as a consequence, to the temperature and light regime that the plants were exposed before and during reproduction and seed development (Pfahler, 1991b). Growth chambers tests indicated that seedling vigor of many generations of Poaceae has been related to seed weight (Rogler, 1954).

The purpose of this study was to examine the effect of seed production site, genetic background and isoline on seed weight and seedling vigor as related to coleoptile, seedling and first leaf length. Variance component estimates, heritability percents and correlation coefficients were calculated.

Materials and Methods

Seeds from four dwarf [tall or normal height (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] isolines (I) in two different genetic backgrounds (Marfed-spring, Burt-winter) were produced at two geographical locations or production sites in the USA: 1. Pullman, Washington in 1989 in the field and; 2. Quincy, Florida in 1995 in pots in the greenhouse. After harvest, the seeds were stored at -20 C.

In this study, seeds from the different production sites (PS) and genetic background (GB) combinations were germinated in the dark at three germination temperatures (GT). Seeds were placed in 16.5 x 17.8 cm plastic growth pouches (Mega international of Minneapolis, MN 55416) with 25 ml of deionized H_2O after being soaked for 15 h at 2 C in deionized H_2O plus 0.2 ml L^{-1} Vitavax ® 200 (Gustafson, McKinney, TX 75070), fungicide for fungi control. Seeds were oriented in germination paper with their embryos pointed down to ensure straight shoot and root growth. Pouches were placed in the vertical partitions of closed transparent plexiglass containers with 100% relative humidity during the germination process. Each treatment was repeated in 3 growth pouches per container (total 30 seeds per treatment) for a total of 16 treatments in each temperature.

The same procedure was repeated for each temperature (10, 18, 26 C) and the experiments were repeated on each of two dates. Measurements at 10, 18 and 26 C were made at 25, 9 and 6 days respectively.

The coleoptile, seedling (coleoptile + first leaf) and first leaf length of 15 seedlings in each treatment combination were measured in millimeters (mm). Appropriate analyses of variance using the general linear models procedure (SAS Inst., 1988) were performed. The minimum differences for significance presented in the tables were obtained using the Duncan's multiple range values for the maximum number of means to be compared (Harter, 1960).

For seed weight, the variance components, genotypic variance ($\sigma^2_G=\sigma^2_I+\sigma^2_{GB}+\sigma^2_{IxGB}$), environmental variance ($\sigma^2_E=\sigma^2_{PS}$) and genotype x environmental variance ($\sigma^2_{GE}=\sigma^2_{IxPS}+\sigma^2_{GBxPS}+\sigma^2_{IxGBxPS}$) were estimated from the analysis of variance assuming the main effects fixed.

For coleoptile, seedling, and first leaf length, the variance components, genotypic variance ($\sigma^2_G=\sigma^2_I+\sigma^2_{GB}+\sigma^2_{IxGB}$), environmental variance ($\sigma^2_E=\sigma^2_{PS}+\sigma^2_{GT}+\sigma^2_{PSxGT}$) and genotype x environment variance ($\sigma^2_{GE}=\sigma^2_{IxPS}+\sigma^2_{GBxPS}+\sigma^2_{IxGBxPS}+\sigma^2_{IxGT}+\sigma^2_{GBxGT}+\sigma^2_{IxGBxGT}+\sigma^2_{PSxGT}+\sigma^2_{IxPSxGT}+\sigma^2_{GBxPSxGT}+\sigma^2_{IxGBxPSxGT}$) were estimated from each analysis of variance assuming the main effects were fixed. The environmental component included either all five environments (two production sites, three germinating temperatures) or only the two production sites when the germinating temperature is identified.

Broad sense heritability (h^2_B) percentages were calculated using the formula $h^2_B=[\sigma^2_G/(\sigma^2_G+\sigma^2_E+\sigma^2_{GE})]100$. The standard errors of the heritability values were determined by the method of Gordon et al. (1972).

Correlation coefficients (*r* values) indicating the relationship between seed weight, coleoptile, seedling and first leaf length in each of the three GT were calculated.

Results

Seed Weight

The analysis of variance is presented in Table 5-1. All main effects and interactions except IxPS were significant at the 1% level.

Table 5-1. Analysis of variance of the seed weight data in the seed production site study. Fifty seeds of four dwarf isolines in each of two genetic backgrounds were produced at each of two production sites and were weighed individually.

Source of variation	df	Mean square
Isoline (I)	3	3317.11**
Genetic background (GB)	1	5341.28**
IxGB	3	338.39**
Production site (PS)	1	9197.14**
IxPS	3	104.73
GBxPS	1	483.19**
IxGBxPS	3	313.52**
Error	784	68.72

** F value significant at the 1% level, respectively.

The means are presented in Table 5-2. The means ranged from 17.00 to 40.66 mg per seed. The heaviest seeds were produced in Q95, regardless of isoline or GB, indicating the importance of PS. In general, Marfed was consistently heavier than Burt and T-0 was heavier than SD-1, SD-2 and D-12. No significant differences were observed among the isolines, except D-12 which was significantly lighter.

The variance component estimates and the heritability percent are shown in Table 5-3. The genetic factors I, GB, and the environmental factor PS were the major contributors to the total variance, with percentages of 26.08, 21.17 and 36.65%, respectively. The heritability percent of 52 could be considered moderate with the main effects, I and GB, being the major contributors to the genetic variance.

Coleoptile Length

The analysis of variance is shown in Table 5-4. The main effect, I, PS and GT and most of their interactions were significant at the 1% level. Although the main effect GB was not statistically significant, the significance of the GB with other main effects indicated its importance.

The means are presented in Table 5-5 and ranged from 41 to 105 mm. Regardless of GT, PS or GB, T-0 was consistently longer than SD-1, followed by SD-2, and D-12. Although GB means in general were not different, the effect of PS was clearly observed. Q95 was significantly longer than P89 in

Table 5-2. Individual seed weight means from the seed production site study. Fifty seeds from each of four dwarf isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in two genetic backgrounds (Marfed, Burt) produced at two seed production sites [Pullman 1989 (P89), Quincy 1995 (Q95)], were weighed individually.

Isoline	Genetic background	Seed production site	Mean
			--mg--
T-0	Marfed	P89	23.89†
		Q95	40.66
	Burt	P89	26.42
		Q95	30.30
SD-1	Marfed	P89	25.50
		Q95	32.37
	Burt	P89	21.03
		Q95	30.01
SD-2	Marfed	P89	26.20
		Q95	34.45
	Burt	P89	21.42
		Q95	22.81
D-12	Marfed	P89	19.74
		Q95	26.19
	Burt	P89	17.00
		Q95	23.67

† Minimum differences among any combination of isoline, genetic background, seed production site and temperature means were 4.01 and 5.17 at the 5 and 1% level, respectively.

Table 5-3. Variance components estimates (percent of each component to the total variance) and heritability (h^2_B) percent ± standard error for seed weight in the seed production site study.

Variance component	Estimate
σ^2_I	16.24** (26.08)
σ^2_{GB}	13.18** (21.17)
σ^2_{IxGB}	2.70** (4.34)
σ^2_{PS}	22.82** (36.65)
σ^2_{IxPS}	0.36 (0.01)
σ^2_{GBxPS}	2.07** (3.32)
$\sigma^2_{IxGBxPS}$	4.90** (7.87)
h^2_B	52±8

** F value significant at the 1% level.

Table 5-4. Mean squares from the analysis of variance of the coleoptile, seedling and first leaf length data in the seed production site study. Seeds of four dwarf isolines in each of two genetic backgrounds were produced at each of two production sites and were measured after germination at three temperatures in the dark.

Source of variation	df	Length		
		Coleoptile	Seedling	First leaf
Isoline (I)	3	53571**	86092**	10099**
Genetic background (GB)	1	53	171	47
IxGB	3	2609**	4554**	874**
Production site (PS)	1	15353**	85689**	29061**
IxPS	3	1318**	7910**	3022**
GBxPS	1	2078**	1412**	47
IxGBxPS	3	11	787*	789**
Germination temperature (GT)	2	132306**	669607**	332550**
IxGT	6	1161**	5698**	4534**
GBxGT	2	223**	48988**	43152**
IxGBxGT	6	127**	1348**	1171**
PSxGT	2	107	4472**	4997**
IxPSxGT	6	157**	422	328
GBxPSxGT	2	57	829*	759*
IxGBxPSxGT	6	99*	561	430
Date (D)	1	227*	370	1042*
IxD	3	108	116	36
GBxD	1	5	2679**	2649**
IxGBxD	3	76	216	101
PSxD	1	24	107	293
IxPSxD	3	188**	664*	1224**
GBxPSxD	1	181*	124	22
IxGBxPSxD	3	47	1206**	1522**
GTxD	2	124	3328**	2432**
IxGTxD	6	104*	335	360
GBxGTxD	2	51	7735**	8583**
IxGBxGTxD	6	24	745**	652**
PSxGTxD	2	21	63	32
IxPSxGTxD	6	77	321	217
GBxPSxGTxD	2	268**	752*	921**
IxGBxPSxGTxD	6	35	570*	393
Error	1344	47	255	220

*,** F value significant at the 5 and 1% level, respectively.

Table 5-5. Coleoptile length means from the seed production site study. Seeds of four dwarf isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in each of two genetic backgrounds (Marfed, Burt) were produced at two seed production sites [Pullman 1989 (P89), Quincy 1995 (Q95)], were measured after germination at three temperatures (10, 18, 26 C) in the dark.

Isoline	Genetic background	Seed production site	Germination temperature 10	18	26
			----------	mm	----------
T-0	Marfed	P89	91†	75	58
		Q95	104	92	72
	Burt	P89	100	86	66
		Q95	105	97	76
SD-1	Marfed	P89	92	64	53
		Q95	97	72	58
	Burt	P89	83	61	51
		Q95	85	65	51
SD-2	Marfed	P89	80	58	46
		Q95	91	67	54
	Burt	P89	82	59	50
		Q95	89	65	50
D-12	Marfed	P89	70	48	42
		Q95	74	56	48
	Burt	P89	68	56	41
		Q95	73	52	42

† Minimum differences among any combination of isoline, genetic background, seed production site and temperature means were 5 and 6 at the 5 and 1% level, respectively.

all I, GB and GT. With increasing temperatures, the means decreased significantly.

The variance component estimates and the heritability percents are shown in Table 5-6. The genetic factor I and the environmental factor GT were the major contributors to the total variance, with percentages of 30.60 and 56.67, respectively. The heritability percent of 33 over combined GT was considered relatively low with the main effect GT contributing slightly over half of the total variance. However, variance component estimates when the effect of GT was not included, showed that the genetic factor I contributed around 70-77% of the total variance. Consequently, the heritability percents at each individual temperature ranged from 79 to 85 which would be considered relatively high.

Seedling Length

The analysis of variance is shown in Table 5-4. The main effects I, PS and GT and most of their interactions were significant at the 1% level. Although the main effect GB was not significant, the significance of the GB with other main effects indicated its importance.

The means are presented in Table 5-7 and ranged from 80 to 216 mm. In general, T-0 was longer than SD-1, which was longer than SD-2, which was longer than D-12. However, Marfed T-0 and SD-1 were not different. It appears that, in

Table 5-6. Variance component estimates (percent of each component to the total variance) and heritability (h^2_B) percent ± standard error for the coleoptile length of the seed production site study. Seeds of four dwarf isolines (I) in each of two genetic backgrounds (GB) were produced at two seed production sites (PS). The coleoptile length was obtained after the germination of these seeds at three temperatures (10, 18, 26 C) in the dark. The variance components were determined over the combined germination temperatures (GT) and each of three temperatures individually.

		Germination temperature (C)		
Variance component	Combined GT	10	18	26
σ^2_{I}	149** (30.60)	144** (74.61)	211** (77.29)	110** (71.90)
σ^2_{GB}	0 (0.00)	1** (0.01)	0 (0.00)	0 (0.00)
σ^2_{IxGB}	14** (2.88)	20** (10.36)	14** (5.13)	11** (7.19)
σ^2_{PS}	21** (4.31)	22** (11.40)	27** (9.89)	15** (9.80)
σ^2_{IxPS}	7** (1.44)	3** (1.55)	12** (4.40)	10** (6.54)
σ^2_{GBxPS}	6** (1.23)	2** (0.01)	8** (2.93)	7** (4.58)
$\sigma^2_{IxGBxPS}$	0 (0.00)	1 (0.00)	1 (0.00)	0 (0.00)
σ^2_{GT}	276** (56.67)			
σ^2_{IxGT}	9** (1.85)			
σ^2_{GBxGT}	0** (0.00)			
$\sigma^2_{IxGBxGT}$	1** (0.00)			
σ^2_{PSxGT}	0 (0.00)			
$\sigma^2_{IxPSxGT}$	2** (0.00)			
$\sigma^2_{GBxPSxGT}$	0 (0.00)			
$\sigma^2_{IxGBxPSxGT}$	2* (0.00)			
h^2_B	33±14	85±10	82±8	79±10

*,** F value significant at the 5 and 1% level, respectively.

Table 5-7. Seedling length means from the seed production site study. Seeds of four dwarf isolines [normal height or tall (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2) and dwarf (D-12)] in each of two genetic backgrounds (Marfed, Burt) were produced at two seed production sites [Pullman 1989 (P89), Quincy 1995 (Q95)] and were measured after germination at three temperatures (10, 18, 26 C) in the dark.

Isoline	Genetic background	Seed production site	Germination temperature 10	18	26
			----------	mm	----------
T-0	Marfed	P89	183†	104	111
		Q95	212	117	152
	Burt	P89	174	115	129
		Q95	207	138	153
SD-1	Marfed	P89	200	99	115
		Q95	216	105	133
	Burt	P89	159	103	128
		Q95	173	108	134
SD-2	Marfed	P89	171	89	100
		Q95	207	102	124
	Burt	P89	153	101	121
		Q95	174	112	126
D-12	Marfed	P89	151	80	97
		Q95	158	84	101
	Burt	P89	127	99	107
		Q95	138	97	118

† Minimum differences among any combination of isoline, genetic background, seed production site and temperature means were 11 and 14 at the 5 and 1% level, respectively.

general, GB means were not different but the effect of PS was clearly observed. Seedling length for Q95 was significantly greater than P89 in all I, GB and GT. The effect of GT was highly significant. The longest seedlings were obtained at 10, followed by 26 and 18 C.

The variance component estimates and the heritability percents are shown in Table 5-8. The genetic factor I and the environmental factor GT were the major contributors to the total variance, with percentages of 11.15 and 65.32, respectively. The heritability percent of 12 over combined GT was considered very low with the main effect GT contributing over 60% to the total variance. On the other hand, variance component estimates at each individual temperature, indicated that the genetic factors I and GB and the environmental factor PS were the major contributors to the total variance. The heritability percents for each individual temperature were 76, 77 and 48 at 10, 18 and 26 C, respectively.

First Leaf Length

The analysis of variance is shown in Table 5-4. The main effects I, PS and GT and most of their interactions were significant at the 1% level. Although the main effect, GB, was not statistically significant, the significance of the GB with other main effects indicated its importance.

Table 5-8. Variance component estimates (percent of each component to the total variance) and heritability (h^2_B) percent ± standard error for the seedling length of the seed production site study. Seeds of four dwarf isolines (I) in each of two genetic backgrounds (GB) were produced at two seed production sites (PS). The seedling length was obtained after the germination of these seeds at three temperatures (10, 18, 26 C) in the dark. The variance components were determined over the combined germination temperatures (GT) and each of three temperatures individually.

Variance component	Combined GT	Germination temperature (C)		
		10	18	26
σ^2_{I}	238** (11.15)	500** (43.25)	137** (48.41)	168** (36.76)
σ^2_{GB}	0 (0.00)	287** (24.83)	66** (23.32)	52** (11.38)
σ^2_{IxGB}	24** (0.00)	93** (8.05)	14** (4.95)	0 (0.00)
σ^2_{PS}	119** (5.58)	216** (18.69)	38** (13.43)	136** (29.76)
σ^2_{IxPS}	42** (1.97)	52** (4.50)	24** (8.48)	56** (12.25)
σ^2_{GBxPS}	3** (0.00)	-2 (0.00)†	-2 (2.00)†	22** (4.81)
$\sigma^2_{IxGBxPS}$	12* (0.01)	8 (0.69)	4 (1.41)	23** (5.03)
σ^2_{GT}	1394** (65.32)			
σ^2_{IxGT}	45** (2.11)			
σ^2_{GBxGT}	203** (9.51)			
$\sigma^2_{IxGBxGT}$	18** (0.01)			
σ^2_{PSxGT}	18** (0.01)			
$\sigma^2_{IxPSxGT}$	3 (0.00)			
$\sigma^2_{GBxPSxGT}$	5* (0.00)			
$\sigma^2_{IxGBxPSxGT}$	10 (0.00)			
h^2_B	12±6	76±12	77±11	48±15

*,** F value significant at the 5 and 1% level, respectively.

† Negative variances assumed zero.

The means are presented in Table 5-9 and ranged from 27 to 119 mm. In general, SD-1 was longer than T-0, and SD-2 was longer than D-12 in both GB, although not significantly. The GB were not significantly different from each other over PS, I, GB and GT. However, in most I and GT, Q95 was significantly longer than P89. The effect of GT was highly significant. The longest first leaves were obtained at 10, followed by 26 and 18 C.

The variance component estimates and the heritability percents are shown in Table 5-10. The environmental factor GT was the major contributor to the total variance, with 66.28%. Consequently, the heritability percent of 3 over combined GT could be considered extremely low. However, the variance component estimates at each individual temperature, showed that the GB was the major contributor to the total variance with percentages of 45.98, 70.67 and 30.37 at 10, 18 and 26 C, respectively. The heritability percents for each individual temperature ranged from 48 to 85 which would be considered low to moderate at the higher temperature and moderate to high, at the cooler temperature.

Seed Weight-Seedling Characteristics Interrelationships

The correlation coefficients are shown in Table 5-11. Highly significant *r* values were obtained between seed weight and coleoptile and seedling length at all temperatures. Highly significant positive *r* values were

Table 5-9. First leaf length means from the seed production site study. Seeds of four dwarf isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in each of two genetic backgrounds (Marfed, Burt) were produced at two seed production sites [Pullman 1989 (P89), Quincy 1995 (Q95)] and were measured after germination at three temperatures (10, 18, 26 C) in the dark.

Isoline	Genetic background	Seed production site	Germination temperature 10	18	26
			----------	mm	----------
T-0	Marfed	P89	92†	29	54
		Q95	108	27	80
	Burt	P89	74	29	62
		Q95	102	41	77
SD-1	Marfed	P89	108	35	62
		Q95	119	33	76
	Burt	P89	76	42	77
		Q95	88	43	82
SD-2	Marfed	P89	91	31	53
		Q95	115	35	69
	Burt	P89	71	42	71
		Q95	85	47	77
D-12	Marfed	P89	81	32	55
		Q95	84	27	53
	Burt	P89	59	43	67
		Q95	65	45	76

† Minimum differences among any combination of isoline, genetic background, seed production site and temperature means were 10 and 13 at the 5 and 1% level, respectively.

Table 5-10. Variance component estimates (percent of each component to the total variance) and heritability (h^2_B) percent ± standard error for the first leaf length of the seed production site study. Seeds of four dwarf isolines (I) in each of two genetic backgrounds (GB) were produced at two seed production sites (PS). The first leaf length was obtained after the germination of these seeds at three temperatures (10, 18, 26 C) in the dark. The variance components were determined over the combined germination temperatures (GT) and each of three temperatures individually.

Variance component	Combined GT	Germination temperature (C)		
		10	18	26
σ^2_{I}	27** (2.59)	124** (23.18)	9** (12.00)	20** (10.47)
σ^2_{GB}	0 (0.00)	246** (45.98)	53** (70.67)	58** (30.37)
σ^2_{IxGB}	3** (0.00)	26** (4.86)	2 (2.67)	13** (6.81)
σ^2_{PS}	40** (3.83)	99** (18.51)	1 (1.33)	60** (31.41)
σ^2_{IxPS}	15** (1.44)	29** (5.42)	2 (2.67)	19** (9.95)
σ^2_{GBxPS}	-1 (0.00)†	-2 (0.00)†	6** (8.00)	3 (1.57)
$\sigma^2_{IxGBxPS}$	6** (0.58)	11 (2.06)	2 (2.67)	18* (9.42)
σ^2_{GT}	692** (66.28)			
σ^2_{IxGT}	36** (3.45)			
σ^2_{GBxGT}	179** (17.15)			
$\sigma^2_{IxGBxGT}$	15** (1.44)			
σ^2_{PSxGT}	20** (1.92)			
$\sigma^2_{IxPSxGT}$	1 (0.10)			
$\sigma^2_{GBxPSxGT}$	4* (0.38)			
$\sigma^2_{IxGBxPSxGT}$	6 (0.58)			
h^2_B	3±9	74±17	85±16	48±18

*,** F value significant at the 5 and 1% level, respectively.
† Negative variances assumed zero.

Table 5-11. Correlation coefficients (*r* values) describing the relationship between seed weight (SW), coleoptile length at 10 C (CL10), coleoptile length at 18 C (CL18), coleoptile length at 26 C (CL26), seedling length at 10 C (SL10), seedling length at 18 C (SL18), seedling length at 26 C (SL26), first leaf length at 10 C (FLL10), first leaf length at 18 C (FLL18) and first leaf length at 26 C (FLL26)in the seed production site study.

	Character correlated								
Character	CL10	CL18	CL26	SL10	SL18	SL26	FLL10	FLL18	FL26
SW	0.715**	0.651**	0.681**	0.821**	0.443	0.657**	0.778**	-0.408	0.361
CL10		0.924**	0.936**	0.871**	0.806**	0.804**	0.671**	-0.252	0.361
CL18			0.983**	0.711**	0.875**	0.809**	0.478	-0.267	0.326
CL26				0.754**	0.833**	0.810**	0.536*	-0.318	0.307
SL10					0.527*	0.610*	0.949**	-0.377	0.229
SL18						0.897**	0.276	0.232	0.623**
SL26							0.403	0.175	0.805**
FLL10								-0.409	0.113
FLL18									0.612*

*,** *r* value significant from zero at the 5 and 1% level, respectively.

obtained between coleoptile length and seedling length at all temperatures.

Discussion

The results of this study indicated that variation in seed weight, coleoptile, first leaf and root length was present between isolines in two genetic backgrounds as a result of seed production sites. This variation is the result of many interacting factors (genetic backgrounds, the presence of dwarf alleles in the isolines, seed development and maturation conditions before harvest, and germination temperatures) which are known to influence seed weight and seedling growth (Bewley and Black, 1984; Harrington, 1972; Heydecker, 1977; Miralles and Slafer, 1995, 1996; Allan, 1980b, 1989, Allan et al., 1959). The impact of environmental factors on seed development and its resulting effect on the seedling characteristics is essential to properly assess variation and selection potential.

The results of this study indicated that the two types of environment (production site, germination temperature) had a different impact on the seeds and the resulting seedlings. Production site influenced seed weight while germination temperature had a greater effect on seedling characteristics. For example, Marfed and Burt seeds produced in Quincy greenhouse were heavier than seeds of the same genetic backgrounds produced in Pullman field. Since the parent plants were in the greenhouse in Quincy while in Pullman they were in the field, the stresses associated with plant production were considerably different and difficult to quantify adequately. In general, greenhouse conditions in terms of optimal nutrient, water and pest control levels are less stressful for plant and seed production than field conditions. Nevertheless, seed weight was found to be closely related to production site and genetic background as shown by the relative contributions of each factor to the total variance.

Coleoptile, seedling, and first leaf lengths were most effected by the germination temperatures. Coleoptile and seedling lengths were highly correlated to seed weight. Parodi et al. (1970) reported that heterosis for coleoptile length was probably related to the heavier seed size obtained from hand crosses. These seedling characteristics were primarily influenced by production site but not differences in genetic background. As expected, the isoline variance for coleoptiles was very high because dwarfing and seed weight greatly influenced the coleoptile length. Basically, differences in isoline seed weight due to dwarfing allele presence or absence are reflected in the length of the coleoptile which impacts the length of the seedling. The contribution of production site and genetic background to coleoptile length was low or nonexistent. Seedling length was moderately influenced by isoline, genetic background and production site. First leaf length was highly affected by genetic background, moderately by isoline, and moderately-to-low by production site. These findings are confirmed by the lack of correlations between first leaf length and the other seedling characteristics. Overall, the results indicated that the importance of production site or genetic background depends on the seedling trait being considered.

Germination temperature was the most important factor in the expression of seedling characteristics. Increasing temperatures produced a decrease in coleoptile length indicating that temperature is modifying some physiological processes controlling the coleoptile length. These modifications probably allow the coleoptile cells to extend their walls in the cooler temperatures. Although coleoptile length is related to both cell length and number, only cell elongation is inhibited at high temperature (Allan et al., 1962).

The longest leaves and seedlings were observed at 10 C, as was the case for coleoptiles. However, both leaves and seedlings were in general longer at 26 C than at 18 C. A plausible explanation would be that different mechanisms of cell elongation are involved. At cooler temperatures, the mechanism present in leaves is probably identical to the one in the coleoptile. However, at higher temperatures (26 C), a different mechanism might be operational resulting in longer leaves and seedlings at more moderate temperatures. This observation is supported by the extremely high value for the environmental variance observed in seedling and first leaf length. In summary, this study indicated that the expression of the dwarfing character, which is inherited qualitatively but is associated with many physiological functions, can be considerably altered by environmental factors. In this study, two environmental factors, production site and germination temperature, which could influence seed and seedling characters were examined. In general, the germination temperature was a more dominant factor. Apparently, the temperature conditions at planting would be more important than seed source especially in relation to the stand establishment problem caused by short coleoptile length.

CHAPTER 6
THE EFFECT OF VARIOUS LIGHT TREATMENTS ON SEEDLING CHARACTERISTICS OF DWARF ISOLINES

Introduction

The coleoptile, which is a subterranean organ under normal conditions, develops below the soil surface in the dark. Since exposure to light during coleoptile elongation is known to reduce final coleoptile length (Lawson and Weintraub, 1975), information on the effect of light on final coleoptile length would be desirable in predicting emergence under several soil types and soil moisture conditions.

This study examined the effect of various light treatments during germination on the coleoptile and total seedling length of four homozygous dwarf isolines in two genetic backgrounds.

Materials and Methods

Seeds from four homozygous dwarf isolines (I): [normal height = T-0 ($rht_1rht_1rht_2rht_2$); semidwarf-1 = SD-1 ($Rht_1Rht_1rht_2rht_2$); semidwarf-2 = SD-2 ($rht_1rht_1Rht_2Rht_2$); dwarf = D-12($Rht_1Rht_1Rht_2Rht_2$)] in each of two different genetic backgrounds (GB) Marfed, a spring cultivar and Burt, a winter cultivar were tested. These seeds were produced in Quincy in 1995 in the greenhouse in pots and stored after harvest at -20 C. The experiment was conducted in a growth chamber at 18 C temperature and 6 light treatments (0, 1, 15, 60, 180, 1440 min d^{-1}). The light sources in the growth chamber were both fluorescent and incandescent bulbs producing a total of 164 µmol $m^{-2}s^{-1}$ of photosynthetically active radiation. For all treatments, seeds were soaked for 15 h at 2 C in deionized water plus 0.2 ml L^{-1} Vitavax ® 200 (Gustafson, McKinney, TX 75070) fungicide for fungi control and then enfolded in germination paper inserted in 16.5 x 17.8 cm plastic growth pouches (Mega International of Minneapolis, Minneapolis, MN 55416) which allowed light transmission and contained 25 ml of deionized water. The seeds were oriented in the germination paper with their embryos down to ensure straight shoot and root growth. Pouches were placed in the vertical partitions of closed transparent plexiglass containers with 100% relative humidity during the germination process. The experiment was replicated on each of two dates. The measurements of the coleoptile and seedling (coleoptile + first leaf) length were taken in millimeters (mm) 10 days after germination initiation.

A complete factorial variance analysis involving all combinations of the main effects, light treatment, genetic background, isoline and date, was performed on each character (SAS Inst., 1988). The minimum differences for significance presented in the tables were obtained using the Duncan's multiple range values for the maximum number of means to be compared (Harter, 1960).

Results

Coleoptile Length

The analysis of variance is presented in Table 6-1. The main effects, GB, L and I as well as all the interactions were highly significant, except IxGB which was only significant at the 5% level.

The means are presented in Table 6-2. The 0 min d^{-1} light treatment represented length in the dark, the control treatment or the 'normal' growth conditions. With 1 min d^{-1} light treatment a highly significant reduction was observed in the length of all isolines in both GB. When 15 min d^{-1} light treatment was used, length decreased further, except for SD-2 and D-12 in Marfed where the significance was at the 5% level. The 60 min d^{-1} light treatment further decreased length. When 180 min d^{-1} light treatment was examined, the length of Marfed T-0 and SD-1 and Burt T-0 and D-12 remained statistically unchanged, while SD-2 and D-12 in Marfed and SD-1 and SD-2 in Burt showed a statistically significant increase. With the 1440 min d^{-1} light treatment, or continuous light, the coleoptiles of all isolines in both

Table 6-1. Mean squares from the analysis of variance on the effect of six light treatments on the coleoptile and seedling length of four dwarf wheat isolines in two genetic backgrounds, germinated at 18 C for 10 d.

Source of variation	df	Length	
		Coleoptile	Seedling
Isoline (I)	3	35919**	254743**
Genetic background (GB)	1	1073**	2732**
IxGB	3	42*	1175**
Light treatment (L)	5	98063**	137772**
LxI	15	2488**	4261**
LxGB	5	832**	1504**
LxIxGB	15	35**	922**
Error	1872	14	230

*,** F value significant at the 5 and 1% level, respectively.

Table 6-2. The effect of six (0, 1, 15, 60, 180, 1440 min d^{-1}) light (fluorescent and incandescent bulbs producing 164 µmol $m^{-2}s^{-1}$) treatments on the coleoptile length mean of four dwarf wheat isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in two genetic backgrounds Marfed, Burt), germinated at 18 C for 10 d.

Genetic background	Isoline	Light treatment (min d^{-1}) 0	1	15	60	180	1440
		mm					
Marfed	T-0	98†	64	54	31	30	43
	SD-1	72	45	40	26	26	32
	SD-2	67	41	39	24	27	30
	D-12	56	34	32	21	23	26
Burt	T-0	97	56	48	31	31	42
	SD-1	72	37	34	25	29	33
	SD-2	67	37	32	26	29	32
	D-12	56	32	28	23	22	28

† Minimum differences among any combination of genetic background, isoline, and light treatment means at 5 and 1% level were 2 and 3, respectively.

GB, showed a highly significant increase in their length. It was also observed that under continuous light treatment, the seedlings in all isolines and genetic backgrounds germinated (radicle protruding) at least 2 days earlier.

Seedling Length

The analysis of variance is presented in Table 6-1. All sources of variation were significant at the 1% level.

The means are presented in Table 6-3. The 0 min d^{-1} light treatment represented seedlings growing in the dark or the control treatment. With 1 min d^{-1} light treatment, seedling length remained unchanged in Marfed T-0 and Burt T-0, SD-1 and SD-2. In Marfed SD-1, SD-2 and D-12, and Burt D-12 a significant decrease in seedling length was observed. When 15 min d^{-1} light treatment was examined, seedling length of all four isolines and GB remained unchanged. In both GB, T-0 length decreased significantly with 60 min d^{-1} light treatment, however, the remaining isolines did not change with the exception of Marfed D-12 which showed a significant increase in seedling length. With 180 min d^{-1} light treatment, seedling length of all isolines and GB increased significantly with the exception of D-12. With 1440 min d^{-1} light treatment, the seedling length was not only significantly longer than the seedlings at 180 min d^{-1} light treatment but they were also significantly longer than the seedlings grown in the dark (0 min d^{-1}).

Table 6-3. The effect of six (0, 1, 15, 60, 180, 1440 min d^{-1}) light (fluorescent and incandescent bulbs producing 164 µmol $m^{-2}s^{-1}$) treatments on the seedling length means of 4 dwarf wheat isolines [normal height or tall (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in two genetic backgrounds (Marfed, Burt), germinated at 18 C for 10 d.

Genetic background	Isoline	Light treatment (min d^{-1})					
		0	1	15	60	180	1440
		mm					
Marfed	T-0	152†	148	144	130	149	220
	SD-1	127	115	109	112	121	163
	SD-2	123	102	106	110	121	159
	D-12	103	86	84	94	98	124
Burt	T-0	141	146	155	136	147	204
	SD-1	122	116	115	112	127	177
	SD-2	121	115	116	110	124	164
	D-12	106	96	93	91	95	127

† Minimum differences among any combination of genetic background, isoline, and light treatment means at the 5 and 1% level were 8 and 11 respectively.

Discussion

In the dark (0 min d^{-1} light treatment), the coleoptile means were considerably longer than at any other light treatment as would be expected with a subterranean organ, such as the coleoptile (Lawson and Weintraub, 1975; Wiedenroth et al., 1990). As the light treatment increased in duration, from 0 min to 180 min d^{-1}, means of the coleoptiles decreased in length, indicating that the coleoptile elongation is negatively associated with light treatment. This length reduction with an increase in light treatment was reported previously (Pinthus and Abraham, 1996; Bleiss et al., 1987; Bleiss and Ludwig, 1990). When continuous light (1140 min d^{-1}) was used the coleoptile means were significantly longer than those at 180 min d^{-1} in all isolines and GB, however, the increase was more pronounced in T-0 than in the other isolines. Under continuous light, coleoptile and first leaf elongated at the same rate. The tip of the coleoptile is penetrated by the first leaf between 72 and 84 h in light and between 144 and 168 h in the dark at 25 C (Wiedenroth et al., 1990). The increase in growth is almost entirely restricted to the apical section of the coleoptile (Lawson and Weintraub, 1975).

It was observed that with continuous light, the seeds germinated at least 2 days earlier than in any other light treatment and that the coleoptiles turned green (instead of the normal yellowish color) as if they were leaves. The coleoptiles of Poaceae which are by nature a subterranean organ are 'transformed' into a 'leafy-like' photosynthetic organ when germinated in continuous light. Actually, Wiedenroth et al. (1990) reported that, during ontogenesis the coleoptile is the first organ capable of photosynthesis and, in fact, contains chlorophyll. It is well known that the phytochrome is the main photoreceptor pigment which controls the growth of the coleoptile, but the transduction chain between phytochrome photoconversion and extension growth regulation, however, is still a matter of debate (Bleiss et al., 1987; Bleiss and Ludwig, 1990).

Increases in light treatment up to and including 60 min d^{-1} sharply reduced coleoptile length in all dwarf isolines, with the decrease much more pronounced in the tall isoline. Further increases above 60 min d^{-1} had no effect on the coleoptile length, although in continuous light (1440 min d^{-1}) a slight increase in all isolines was observed, presumably because of chlorophyll production and assimilation which promoted growth. Bleiss and Ludwig (1990) also reported that after the onset of 3 h of red-light, a persistent growth promotion was observed only in the tip region of the coleoptile. Genetic background was an influencing factor in the response of dwarf isolines to light treatment in this study.

The results reported herein of seedling length were not as consistent or predictable as the lengths of the coleoptile. The longest seedlings were observed under continuous light treatment. Also, the longest leaves were observed with this same treatment explaining the observed seedling lengths. In other words, these results indicated that the first leaf length was the most important contributory organ for the total seedling length. Under continuous light, the first leaf of every seedling started photosynthesizing as soon as it emerged from the coleoptile. With continuous light and optimal temperature conditions, a maximum area per leaf can be obtained (Evans et al., 1975).

Light incidence was shown to be a major factor in controlling coleoptile length among dwarf isolines presumably because of the photosensitivity nature of the hormone auxin influencing coleoptile growth and final length. This study showed that final coleoptile length of seedlings in the dark are approximately twice as long as those germinated under continuous light, which essentially is in agreement with Wiedenroth et al. (1990). The effect of red-light on the auxin level in coleoptiles is not yet understood in relation to phytochrome controlled growth (Bleiss and Ludwig, 1987). Apparently, the yield of diffusible auxin decreased following exposure of intact seedlings to red-light, and so, it was assumed that coleoptile elongation followed by light exposure is not due to the effect of auxin. The blue light-induced growth response was often discussed as a basis for the phototropic reaction of coleoptiles to unilateral blue light.

Light transmission and filtering is greatly influenced by soil texture and moisture which could contribute to emergence and stand establishment in dwarf isolines. Light duration is, in fact, a major factor for seedling growth. It influences primarily the growth of the first leaf which starts photosynthesizing as soon as it perforates the soil surface. Further studies linking light transmission and soil characteristics should be conducted to determine if an interaction with dwarf isolines may exist.

CHAPTER 7
THE EFFECT OF GIBBERELLIC ACID AND TEMPERATURE ON THE COLEOPTILE DIMENSIONS OF DWARF ISOLINES

Introduction

Coleoptile length in wheat is associated with both an increased number and length of cells. Cell division was reported to be completed 54 h after germination initiation at 25 C in the dark when the coleoptile length was between 5-10 mm in length (Wright, 1960). Thereafter, increases in length were associated with increasing the longitudinal length of the epidermal cells (Wiedenroth et al., 1990). As a result of this cell division-cell expansion sequence, the length of the epidermal cells of the coleoptiles of 30-35 mm ranged from 890 µm at the base to 1307 µm at the medial segment with width of these cells considerably lower at 18-19 µm. This sequence also resulted in a decrease in the diameter of the fully-elongated coleoptile from the base to the apex.

No information is available concerning the relationship between coleoptile length and diameter. The purpose of this study was to determine the combined effect of temperature, genetic background, isoline and gibberellic acid on the length and cross section dimensions of the coleoptile.

Materials and Methods

Seeds from four homozygous dwarf isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] in two genetic backgrounds (GB) Marfed-spring, and Burt-winter. These seeds were produced under field conditions in 1989 in Pullman-Washington and stored after harvest at -20 C. Seeds of each isoline-genetic background combination were soaked for 4 d at 2 C, in 10 ml of one of five concentrations (0, 5, 50, 100 and 500 mg L^{-1}) of gibberellic acid (GA) plus 0.2 ml L^{-1} Vitavax ® 200 (Gustafson, McKinney, TX 75070) fungicide, for fungi control and enfolded in germination paper in 16.5 x 17.8 cm plastic growth pouches (Mega International of Minneapolis, Minneapolis, MN 55416) containing 25 ml of the same GA solution used in the soaking solution. The seeds were oriented in the germination paper with their embryos down to ensure straight shoot and root growth. The pouches were placed in the vertical partitions of closed transparent plexiglass containers with 100% relative humidity during the germination process. Four pouches, each containing 10 seeds, were used for each isoline-GB-GA solution combination. Each experiment was repeated twice (80 seeds total for every treatment combination). The containers were placed randomly inside a constant temperature (no light) growth chamber. The coleoptile length (mm) of 15 seedlings at 2, 10 and 18 C were measured after 68, 26 and 9 days after germination initiation, respectively. The seedling section containing the coleoptile was then placed in a killing and fixing solution (62 parts H_2O + 5 parts formaldehyde + 3 parts glacial acetic acid + 30 parts glycerin) and stored at 2 C.

Diameter measurements were taken on two segments of each coleoptile: 1. Basal - just above the scutellar node and; 2. Medial - approximately midway between the basal segment and pore. The coleoptile and its appearance in cross-section are shown in Fig. 7-1 and 7-2. Since the cross section is oval, the diameter (µm) of both the broad and narrow axes of both the basal and medical segments were measured at 15x magnification with a calibrated eyepiece micrometer.

An appropriate analysis of variance was performed on each of the five (length, basal-broad, basal-narrow, medial-broad, medial-narrow) characters (SAS Inst., 1988). The minimum differences for significance presented in the tables were obtained using the Duncan's multiple range values for the maximum number of means to be compared (Harter, 1960).

Linear regression analysis between coleoptile length as the dependent variable (*y*) and the various diameters as the independent variable (*x*) were performed. All possible combinations of three germination temperatures, four isolines, two genetic backgrounds and five gibberellic acid concentrations were represented in the 120 data pairs. The analyses included all 120 pairs (118 df) and a subdivision

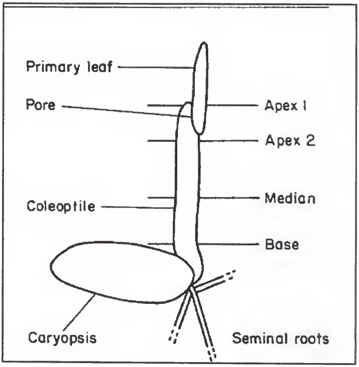

Fig 7-1. A wheat seedling showing the locations of the base or basal (just above the scutellar node) and median or medial (approximately midway between the seed and pore) segments on which the cross-sections were measured (Wiedenroth et al., 1990).

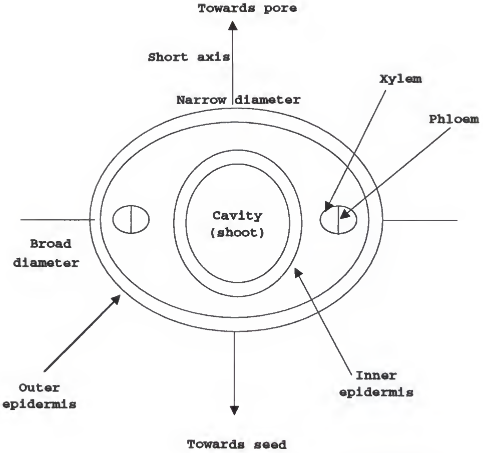

Fig. 7-2. The cross-section of the coleoptile of wheat showing its oval form with the broad and narrow diameter indicated (Wiedenroth et al., 1990).

of the data so that the influence of each variable within each main effect could be determined.

Results

Coleoptile Length

The analysis of variance is presented in Table 7-1. The main effects and all interactions were highly significant at the 1% level.

The means are presented in Table 7-2. The coleoptiles ranged from 57 to 133 mm. In general, the longest coleoptiles were found at 2 C, followed by 10 C and 18 C. The most pronounced and significant increase in length due to the application of GA were also found at 2 C. When comparisons were made between GB and across temperatures, Marfed was more sensitive to the effect of GA and temperature than Burt.

Basal-Broad Diameter

The analysis of variance is shown in Table 7-1. All main effects and interactions except IxT, GBxI and GBxIxT were significant at the 1% level.

The means are presented in Table 7-2. The diameter means ranged from 1182 to 1527 µm. The largest diameters were observed at 10 C, followed by 18 and 2 C. Independent of temperature, Marfed generally had larger diameters than

Table 7-1. Mean squares from the analysis of variance on the effect of 5 gibberellic acid (GA) concentrations in for four dwarf wheat isolines, two genetic backgrounds, three temperatures, on the coleoptile length and diameter measurements of seedlings grown in the dark.

Source of variation	df	Coleoptile length	Segment and direction			
			Basal		Medial	
			Broad	Narrow	Broad	Narrow
Temperature (T)	2	102763**	1252003**	2723446**	3698031**	7530836**
Concentration (C)	4	7044**	56675**	147917**	336353**	550218**
TxC	8	856**	297334**	78076**	79750**	42757**
Isoline (I)	3	98324**	163973**	66401**	30056**	36412**
IxT	6	2268**	1536	24019**	152428**	58276**
IxC	12	862**	34595**	27350**	134297**	75374**
IxTxC	24	307**	29988**	31278**	41968**	69159**
Genetic background (GB)	1	24907**	486000**	860628**	57212**	801167**
GBxT	2	1546**	63577**	651586**	1907692**	535541**
GBxC	8	1520**	35406**	58100**	76314**	182220**
GBxTxC	8	739**	98641**	74797**	129370**	111872**
GBxI	3	1736**	628	44269**	38642**	34409**
GBxIxT	6	1232**	2895	24205**	132000**	40836**
GBxIxC	12	393**	36943**	30773**	60112**	38912**
GBxIxTxC	24	451**	26386**	33300**	45207**	34385**
Error	1680	70	4268	2198	591	2887

**, F value significant at the 1% probability level.

Table 7-2. Means of coleoptile length (CL), basal-broad (B-B), basal-narrow (B-N), medial-broad (M-B) and medial-narrow (M-N) diameter. The effect of all combinations of 5 gibberellic acid (GA) concentrations (0, 5, 50, 100, 500 mg L^{-1}) on 4 dwarf isolines [normal height or tall (T-0), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)] over 3 (2, 10, 18 C) temperatures (T) and 2 (Marfed, Burt) genetic backgrounds (GB), was examined when seeds were germinated and grown in the dark.

T	GB	Isoline	GA	CL	B-B	B-N	M-B	M-N
				mm	µm			
2	Marfed	T-0	0	104	1350	909	1327	886
			5	120	1379	899	1304	882
			50	124	1416	886	1340	839
			100	136	1409	902	1274	810
			500	123	1393	899	1294	856
		SD-1	0	81	1333	879	1373	892
			5	113	1366	889	1231	853
			50	107	1377	886	1162	843
			100	118	1422	902	1304	823
			500	126	1402	905	1307	866
		SD-2	0	92	1389	899	1340	899
			5	106	1445	889	1399	833
			50	108	1327	866	1192	843
			100	95	1393	902	1353	856
			500	123	1373	889	1231	823
		D-12	0	87	1383	912	1340	892
			5	87	1406	889	1320	833
			50	94	1379	882	1314	853
			100	93	1402	915	1356	889
			500	107	1330	866	1274	830
	Burt	T-0	0	96	1455	895	1439	869
			5	110	1389	912	1307	856
			50	114	1373	879	1251	747
			100	104	1271	876	1067	751
			500	121	1389	909	1310	866
		SD-1	0	100	1452	965	1277	922
			5	99	1383	1093	1195	991
			50	103	1241	833	1067	760
			100	92	1314	872	1063	741
			500	94	1182	866	1034	800
		SD-2	0	93	1386	902	1254	833
			5	100	1379	1001	1155	895
			50	103	1277	951	1188	879
			100	91	1185	839	1021	728
			500	93	1281	882	1034	803
		D-12	0	84	1452	1007	1228	951
			5	88	1264	902	1093	843
			50	87	1287	813	1113	751
			100	85	1330	856	1149	787
			500	89	1211	876	988	760

Table 7-2. (Cont'd)

T	GB	Isoline	GA	CL	B-B	B-N	M-B	M-N
				mm	--------------- µm ---------------			
10	Marfed	T-0	0	77	1376	1067	1445	1228
			5	107	1491	942	1376	942
			50	114	1491	988	1360	928
			100	117	1508	994	1281	886
			500	125	1498	912	1251	856
		SD-1	0	78	1366	1067	1356	1211
			5	92	1409	951	1442	988
			50	92	1488	945	1346	912
			100	96	1481	915	1327	997
			500	100	1488	931	1514	968
		SD-2	0	68	1281	886	1297	1090
			5	80	1441	951	1458	948
			50	78	1478	978	1350	1116
			100	84	1462	932	1343	997
			500	83	1472	922	1396	882
		D-12	0	61	1429	1021	1397	988
			5	64	1435	925	1393	909
			50	68	1435	955	1376	938
			100	68	1465	925	1343	955
			500	69	1409	922	1205	899
	Burt	T-0	0	92	1445	915	1551	1179
			5	101	1402	918	1244	1004
			50	95	1514	984	1422	948
			100	96	1416	935	1327	984
			500	103	1478	935	1429	925
		SD-1	0	75	1267	879	1498	1024
			5	73	1396	905	1343	951
			50	75	1462	935	1386	899
			100	77	1495	965	1412	928
			500	80	1481	915	1412	1090
		SD-2	0	74	1188	869	1360	961
			5	76	1458	945	1478	1001
			50	74	1465	961	1429	935
			100	74	1495	961	1396	942
			500	78	1462	928	1399	1047
		D-12	0	63	1225	846	1290	892
			5	61	1468	958	1416	1205
			50	60	1406	879	1379	882
			100	62	1468	971	1409	978
			500	63	1478	948	1389	1044

Table 7-2. (Cont'd)

T	GB	Isoline	GA	CL	B-B	B-N	M-B	M-N
				mm	--------------- µm ---------------			
18	Marfed	T-0	0	92	1442	1188	1473	1146
			5	99	1442	1192	1317	1146
			50	91	1527	1132	1248	1076
			100	104	1508	1080	1235	1053
			500	111	1511	1109	1340	1024
		SD-1	0	85	1475	1277	1330	1159
			5	84	1379	1109	1343	1100
			50	85	1435	997	1284	1106
			100	86	1445	1047	1225	1096
			500	87	1439	1076	1356	1093
		SD-2	0	74	1435	1228	1360	1202
			5	71	1406	1090	1386	1080
			50	72	1416	1040	1294	1119
			100	75	1465	1063	1346	1192
			500	78	1366	994	1277	1070
		D-12	0	61	1409	1034	1317	1142
			5	59	1406	918	1346	1080
			50	61	1432	1113	1248	1126
			100	62	1412	1050	1277	1139
			500	62	1462	1053	1300	1146
	Burt	T-0	0	98	1455	1027	1416	1100
			5	105	1432	974	1294	955
			50	102	1412	938	1330	905
			100	108	1399	928	1323	892
			500	111	1455	1007	1379	938
		SD-1	0	71	1399	1017	1383	1050
			5	75	1406	965	1327	968
			50	74	1416	958	1350	978
			100	72	1468	1004	1485	1067
			500	75	1402	925	1346	922
		SD-2	0	69	1425	1014	1386	1017
			5	68	1409	978	1383	1027
			50	70	1465	971	1383	974
			100	67	1393	1011	1363	1037
			500	60	1402	925	1396	1044
		D-12	0	58	1429	1053	1435	1113
			5	57	1399	951	1435	1076
			50	57	1376	942	1422	1004
			100	58	1455	971	1416	1037
			500	69	1409	915	1445	1034
MSD†			5%	8	61	44	23	50
			1%	10	80	58	30	66

† Minimum differences for significance among any combination of temperature-genetic background-isoline-GA concentration means within each column.

Burt. T-0 Burt diameters were significantly larger than SD-1, SD-2 and D-12, at 2 C. On the other hand, T-0 Marfed diameters were significantly larger than SD-1, SD-2 and D-12, at 18 C.

Basal-Narrow Diameter

The analysis of variance is presented in Table 7-1. All main effects and interactions were significant at the 1% level.

The means are presented in Table 7-2. Diameter means ranged from 813 to 1277 µm. In general, the smallest diameters were observed at 10 C, followed by 2 and 18 C. Burt had the smallest diameters in all temperatures. Comparisons among isolines, showed that SD-2 and D-12 Marfed were significantly smaller than T-0 at 18 C, while the same pattern was observed in Burt, at 2 C.

Medial-Broad Diameter

The analysis of variance is shown in Table 7-1. All main effects and interactions were significant at the 1% level.

The means are presented in Table 7-2. Diameter means ranged from 988 to 1551 µm. In general, the largest diameters were found at 10 C, followed by 18 and 2 C. Burt diameters were larger than Marfed at 10 and 18 C, but, at 2 C, Marfed was significantly larger than Burt. At 2 C, T-0

Burt diameters were significantly larger than SD-1, SD-2 and D-12. On the other hand, at 18 C, SD-1, SD-2 and D-12 diameters were significantly larger than T-0. Similar results were found in Marfed at 10 C. SD-1 and SD-2 were significantly larger than T-0.

Medial-Narrow Diameter

The analysis of variance is showed in Table 7-1. All main effects and interactions were significant at the 1% level.

The means are presented in Table 7-2. Diameter means ranged from 728 to 1228 µm. In general, the smallest diameters were observed at 2, followed by 10 and 18 C. No differences were observed between GB in each temperature, except at 18 C where Burt was significantly smaller than Marfed. It appears that T-0 was not significantly different from the other three isolines, regardless of GB or temperature. In general, diameters at 0 mg L^{-1} GA were significantly larger than those at 500 mg L^{-1}.

Coleoptile Length-Diameter Measurements Interrelationships

The linear regression values are presented in Table 7-3. Over all variables, the negative *b* value increased in magnitude and significance from the base of the coleoptile

Table 7-3. Intercept (*a*), sign (±) and coefficient (*b*) from the linear regression equation ($y=a+bx$) between coleoptile length (CL) as the dependent variable (y) and the diameter of various coleoptile characters [basal-broad (B-B), basal-narrow (B-N), medial-broad (M-B), medial-narrow (M-N)] as the independent variable (x). All possible combinations of 3 germination temperatures (2, 10, 18 C), four isolines [normal height or tall (T-O), semidwarf-1 (SD-1), semidwarf-2 (SD-2), dwarf (D-12)], two genetic backgrounds (Marfed, Burt) and five gibberellic acid (GA) concentrations (0, 5, 50, 100, 500 mg L^{-1}) were represented in the 120 data pairs. The analysis included all 120 pairs (118 df) and a subdivision of the data so that the influence of each variable within each main effect could be determined.

		Comparisons			
	df	CL vs. B-B	CL vs. B-N	CL vs. M-B	CL vs. M-N
		---------- *a* ± *b* ----------			
All variables	118	99.73-0.009	135.09-0.050*	161.69-0.056**	167.18-0.083**
T-0	28	129.94-0.016	170.48-0.066**	184.15-0.058*	171.39-0.068**
SD-1	28	120.89-0.023	128.43-0.041	157.99-0.053*	149.00-0.062**
SD-2	28	148.71-0.047	160.26-0.081*	176.24-0.071**	168.45-0.089**
D-12	28	210.56-0.100**	175.81-0.111**	165.29-0.071**	152.76-0.084**
Marfed	58	67.85+0.016	160.44-0.071**	182.26-0.069	190.05-0.101**
Burt	58	132.95-0.035	131.69-0.051	156.92-0.056**	160.53-0.082**
2	38	17.64+0.063	99.33+0.003	64.14+0.031	123.48-0.025
10	38	-37.41+0.083*	49.13+0.035	125.14-0.031	118.53-0.037
18	38	-199.40+0.194**	30.21+0.046	175.29-0.072	145.23-0.063
0	22	2.40+0.056	91.12-0.011	81.53-0.001	113.43-0.032
5	22	167.77-0.057	99.60-0.012	208.45-0.091*	184.37-0.100**
50	22	171.27-0.059	172.33-0.089	192.17-0.080*	174.57-0.093**
100	22	146.91-0.041	186.75-0.104	170.06-0.063	167.79-0.085**
500	22	104.51-0.008	144.89-0.055	123.83-0.024	193.12-0.108**

*,** F values significant at the 5 and 1% level, respectively.

(basal diameter) to the median area (medial diameter). However, with increasing temperatures, the positive *b* values also increased in magnitude and significance.

Isolines, genetic backgrounds and GA concentrations, except 0 mg L^{-1} GA, had negative and significant *b* values which increased in magnitude and significance from the base to the medial area of the coleoptile.

In general, the significance of the *b* values became more evident in the medial area of the coleoptile, especially the M-N diameter. Isolines, GB, and all GA concentrations were negatively correlated with the coleoptile length at the 1% level.

Discussion

Coleoptile length in wheat is associated with cell division immediately after germination initiation, followed by increases in longitudinal length of the epidermal cells (Wright, 1960; Wiedenroth et al., 1990). Apparently, almost all of the coleoptile elongation is the result of longitudinal cell expansion and not cell division.

The length of outer epidermal cells of coleoptiles 33 mm in length, was reported to be 890 µm in the base or basal segment to 1307 µm in the median or medial segment (Wiedenroth et al., 1990). The range of coleoptile length in the study reported herein was from 57 to 133 mm indicating that the length of the epidermal cells in the base may range from 1537 to 3587 µm and in the medial area, from 2258 to 5267 µm. Attempts were made in this study to measure the longitudinal length of the epidermal cells microscopically in order to relate cell length to coleoptile length. However, the extreme length of the cells made such measurements impossible. The coleoptile diameters should be negatively associated with coleoptile length if longitudinal cell length increases are positively related to coleoptile length. Therefore, the relationship between coleoptile length and diameter was examined.

The results of this study indicated that, as the coleoptile elongates, the medial diameter became smaller, while changes in the basal area were minimal. More specifically, the medial-broad and medial-narrow diameters of the coleoptile are reduced considerably as the coleoptile lengthens. On the other hand, the basal-broad and basal-narrow diameters of the coleoptile and the reported *b* values indicated that elongation of the cells in the basal area, are not the major contributors to total coleoptile length. The two vascular tubes composed of phloem and xylem which are located between the inner and outer epidermal cells on each side of the coleoptile hollow and extend along the main axis (broad diameter) of the coleoptile (Fig. 7-2). These elements are physically responsible for less reduction in the broad diameter of both the basal and medial area as the coleoptile elongates.

In conclusion, this study confirms that most of the coleoptile elongation is the result of longitudinal cell wall expansion rather than cell division and the effect on diameter increases from the bases to the apex. The differences in coleoptile length due to isoline, genetic background and GA application were also clearly seen in the coleoptile means and in the magnitude and significance of the *b* values moving from the base to the apex.

CHAPTER 8
SUMMARY

This study examined the effect of a number of factors on germination and seedling characteristics of four homogenous and homozygous dwarf isolines [normal or tall height ($rht_1rht_1rht_2rht_2$) = T-0, semidwarf-1 ($Rht_1Rht_1rht_2rht_2$) = SD-1, semidwarf-2 ($rht_1rht_1Rht_2Rht_2$) = SD-2, dwarf ($Rht_1Rht_1Rht_2Rht_2$) = D-12] in two genetic backgrounds ('Marfed' - a spring cultivar, 'Burt' - a winter cultivar).

The reported gibberellic acid (GA) insensitivity caused by the presence of either the dominant Rht_1 or Rht_2 alleles was found to be partially overcome by lower germination temperatures and high GA concentrations (500 mg L^{-1} GA) in the germination medium. The highly significant interaction between temperature, isoline, and GA concentration suggests that the reported coleoptile and first leaf GA-insensitivity in dwarf wheat can be partially overcome by the combination of low temperatures and high GA concentrations. Apparently, the physiological action of the dominant dwarfing alleles is capable of modification by environmental and genetic factors. This study shows that the exogenous application of growth regulators as a seed treatment may be effective in at least partially overcoming the reduced coleoptile length associated with dwarfing in wheat.

The addition of at least two other compounds [100 mg L^{-1} $Ca(NO_3)_2 \bullet 4H_2O$, 96.3 g L^{-1} polyethylene glycol 8000 (-0.156 Mpa osmotic potential)] to 500 mg L^{-1} GA in the germination medium resulted in a significant increase in coleoptile and first leaf length in T-0, SD-1 and SD-2 compared to 500 mg L^{-1} GA alone. Apparently, a number of compounds either singly or in combination will increase coleoptile growth, especially in T-0 and SD-1. This suggests that possibly a preplanting seed treatment could be used to improve stands, especially in T-0 and SD-1. Since the study reported herein evaluated these compounds when present in the germination medium, further studies testing seed treatment under field conditions will be necessary.

Heritability studies indicated that environments in the form of seed production sites and germination temperatures were major factors influencing the expression of seedling characters. This study indicated that the expression of the dwarfing character, which is inherited qualitatively but is associated with many physiological functions, can be considerably altered by environmental factors. In this study, two environmental factors, production site and germination temperature, which could possibly influence seed and seedling characters were examined. In general, the germination temperature was a more dominant factor.

Apparently, the temperature conditions at planting are more important than seed source especially in relation to the stand establishment problem caused by short coleoptile length.

The response of the four isolines in each genetic background to light treatments differed. Light transmission and filtering is greatly influenced by soil texture and moisture which could contribute to emergence and stand establishment in dwarf isolines. Light duration is, in fact, a major factor for seedling growth. It influences primarily the growth of the first leaf which starts photosynthesizing as soon as it emerges from the soil surface. Further studies linking light transmission and soil characteristics should be conducted to determine if an interaction with dwarf isolines may exist.

A highly significant negative relationship between coleoptile length and diameter was found. In conclusion, this study confirms that most of the coleoptile elongation is the result of longitudinal epidermal cell wall expansion rather than cell division and the effect on diameter increases from the bases to the apex. The differences in coleoptile length due to isoline, genetic background and GA application were also clearly seen in the coleoptile means and in the magnitude and significance of the *b* values from the base to the pore.

The results suggested that the problem of shorter coleoptile length in dwarfs can be at least partially overcome by genetic selection, preplant seed treatment with various compounds or a combination of both.

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BIOGRAPHICAL SKETCH

Maria Joao Pereira was born on March 25, 1963, in Maputo, Mozambique. She attended secondary school in Maputo and then a pre-university course. She enrolled for a B.Sc. (agronomy with honors) degree at the University Eduardo Mondlane in 1984 and graduated it in 1988.

After graduation, she worked as a maize breeder at the National Institute of Agronomic Research (INIA) in Maputo. In 1990, she participated in a four month training course on maize improvement at the International Maize and Wheat Improvement Center (CIMMYT), in Mexico City, Mexico.

She entered the Master's program in the Agronomy Department at the University of Florida in May 1991 and graduated with a Master of Science (M.S.) Degree in May 1993. The title of her thesis was "Tomato Spotted Wilt Virus in Peanut: Screening Technique and Assessment of Genetic Resistance Levels."

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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